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CLINICAL AND EXPERIMENTAL

VARIATIONS IN REACTION OF DIFFERENT PARTS OF THE CENTRAL NERVOUS SYSTEM AS INFLUENCED BY DEPRESSANT AND STIMULATING DRUGS*

D. E. JACKSON, PH.D., M.D., CINCINNATI, OHIO

NEARLY forty years ago Cushny emphasized the fact that if two animals be poisoned, one with morphine, the other with chloral to a degree which would just prevent the appearance of spontaneous movements in each, then the spinal reflexes of the animal which had received the morphine would be much more active and more easily elicited than would be the case with those of the animal under the influence of the chloral. It seems certain that some such similar variations as this extend throughout the central nervous system whenever the action of any two drugs which act thereon are compared. My attention was again especially attracted to this phenomenon many months ago when I was observing the action of certain drugs on the respiratory center.

This is a very old subject in pharmacology and a great many papers bearing on one or more phases of the problem have appeared in the literature. It is impossible for me to cite all of these here but I may refer briefly to the excellent papers by Tatum, Waters, Maloney and Fitch (bibliography),[1] Camp,[2] Barlow[3] and his colleagues, Nielsen, Higgins and Spruth,[4] Swanson[5] and his coworkers, Behrens and Reichelt,[6] and Eddy.[7]

The specific depressant action of morphine on the respiratory center has long been known. But one does not ordinarily think of such drugs as ether or chloroform as possessing this specific action, because the depression pro-

*From the department of Pharmacology of the University of Cincinnati Medical School.

duced by these drugs appears to be so evenly distributed over the whole central nervous system. On the other hand the tendency of morphine to mildly excite the cord centers and a few higher nuclei (markedly developed in the

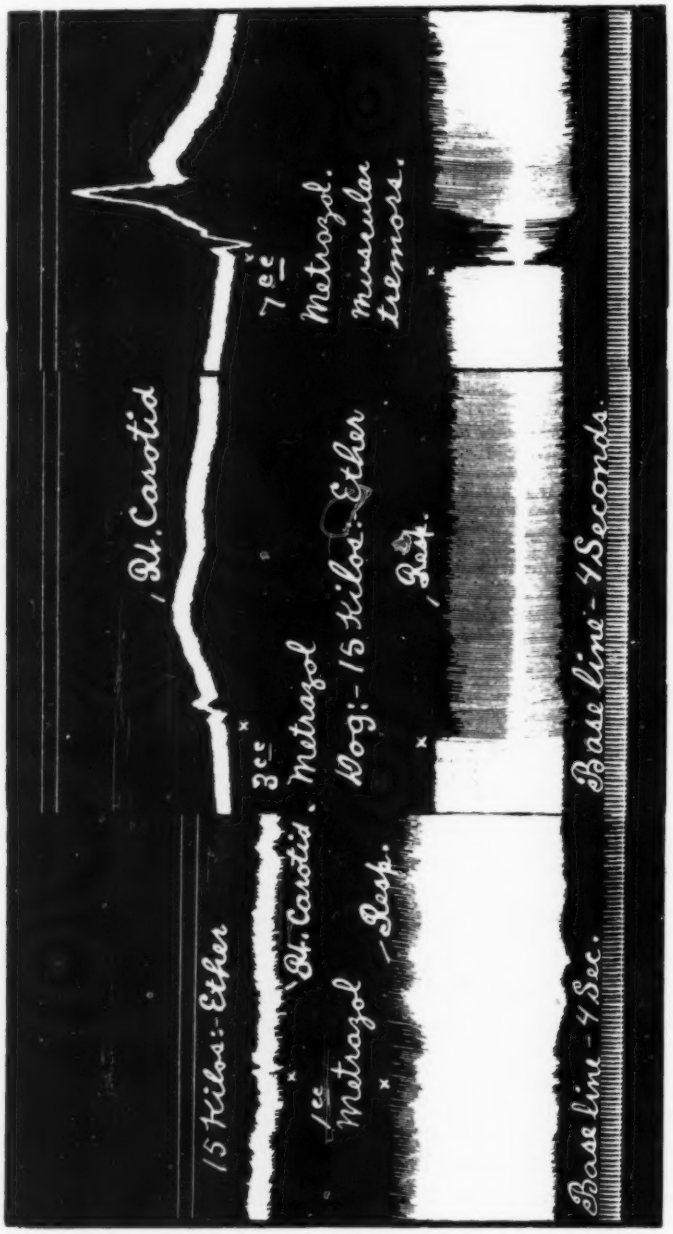


Fig. 1.—For discussion see text. This illustration is made up of three sections all taken consecutively from the same experiment and mounted together. They show the effects of increasing dosage of metrazol on an *etherized* dog.

felines and in frogs in the second stage of poisoning) is practically never thought of in connection with the pharmacologic action of ether or chloroform. Nevertheless these varying degrees of stimulation and depression of all the various centers and tracts of the whole brain and cord are undoubtedly

always present when drugs are acting on the central nervous system. A year or so ago I became impressed with the therapeutic importance of an example of this type of reaction which I observed experimentally in the laboratory.

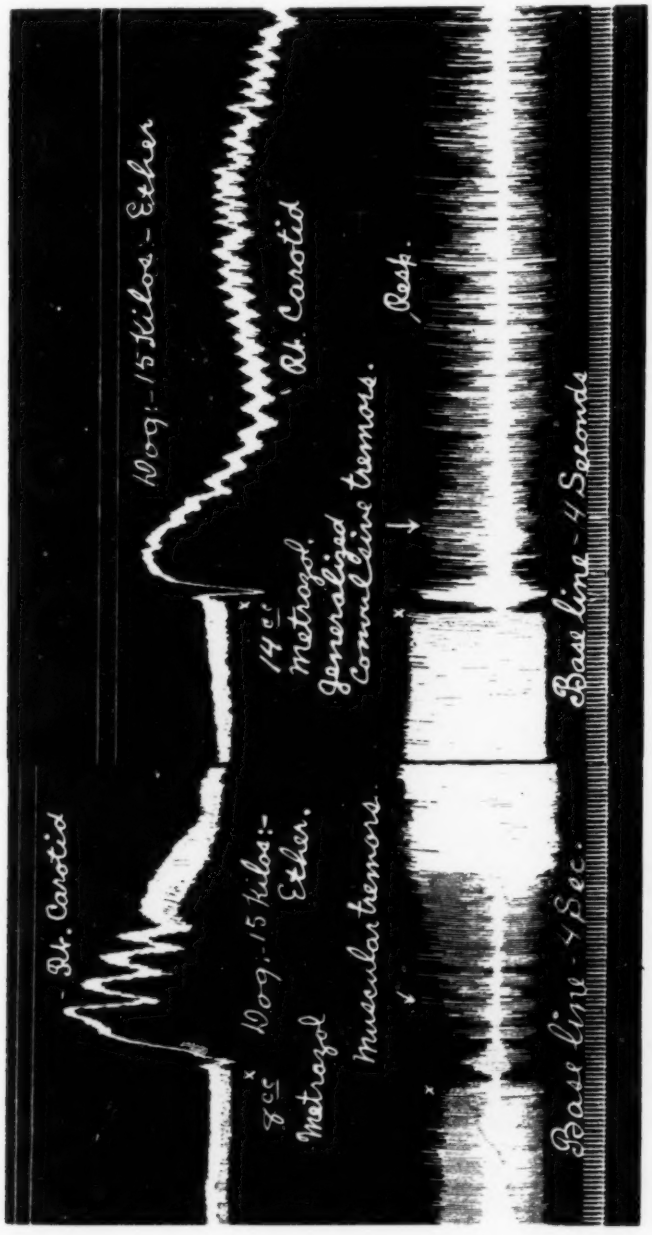


Fig. 2.—Two sections of tracings from the same experiment mounted together. For discussion see text.

This was the failure of metrazol (cardiazol) to produce any worth-while stimulation of the respiratory center in dogs when deeply anesthetized with ether. This observation had also been reported earlier by Camp who obtained similar results with chloroform and morphine. On the other hand Camp and

many others have noted the fact that fair-sized doses of metrazol produce convulsions in animals deeply anesthetized with ether.

All of the experiments described in this paper have been carried out on dogs. I have tried many times to see if I could produce any therapeutically useful stimulation of the respiratory center with metrazol in dogs anesthetized with ether. The lighter the anesthesia the more likely is the metrazol to pro-

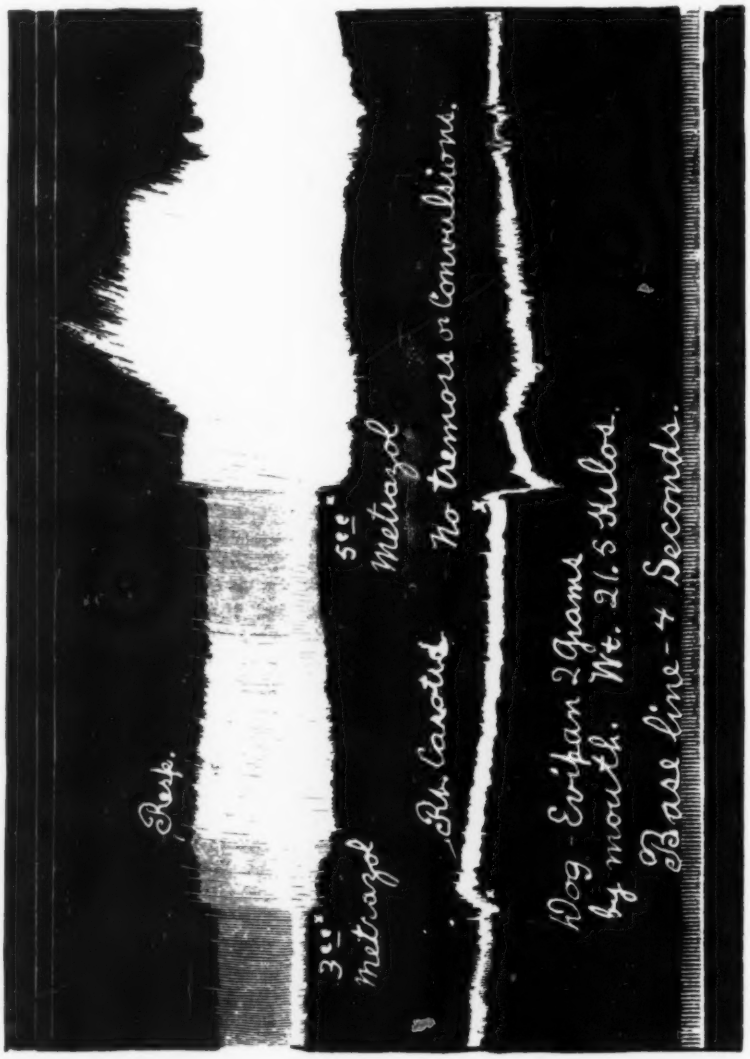


Fig. 3.—This tracing shows the action of metrazol on a dog anesthetized with evipal (evipan).

duce obvious results. And with a very light anesthesia I have occasionally seen a mild respiratory stimulation produced by metrazol. But as the anesthesia is deepened the likelihood that metrazol, if injected intravenously, will specifically stimulate the respiratory center rapidly disappears, and only the probability remains that adequate doses will produce tremors or twitchings or generalized convulsions which appear to come from both the medulla and the cord (Figs. 1 and 2). This would seem to render metrazol useless in many

instances in which a respiratory stimulant is most needed. Two questions which I have not been able to answer satisfactorily arise in this connection, viz., first, does metrazol act (or fail to act) on the human respiratory center when it is deeply depressed by ether in the same way that the drug acts on the dog respiratory center under similar circumstances, and second, if a failing patient continues to breathe feebly after administration of ether has been

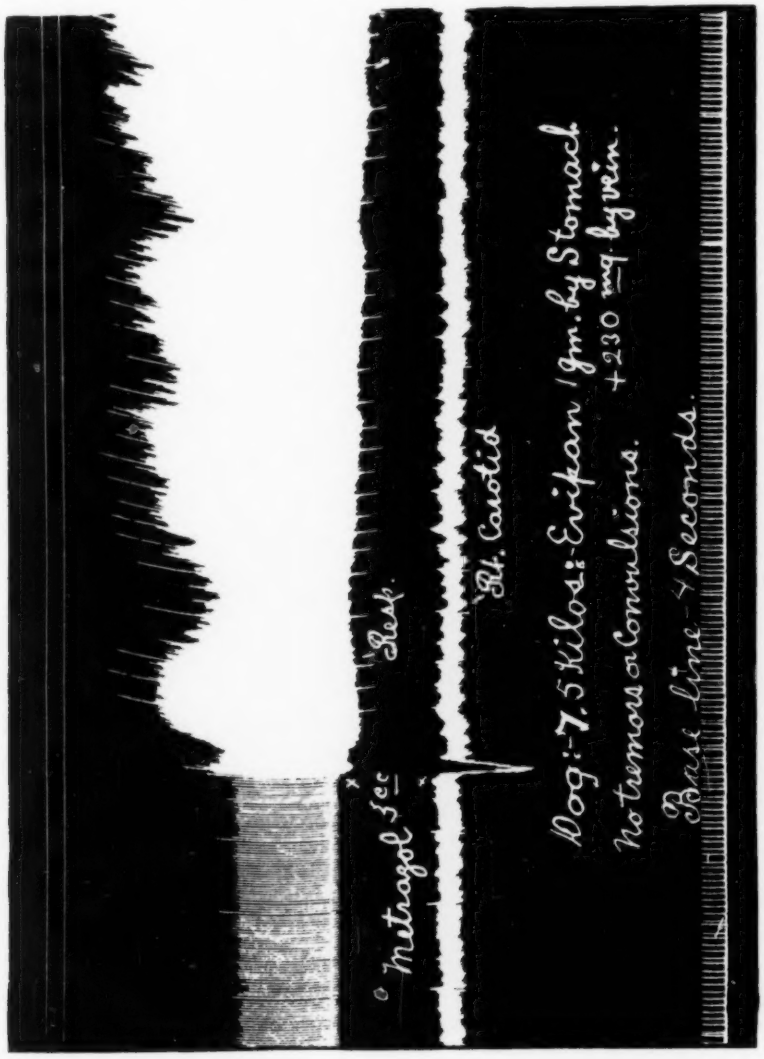

Metrazol 5 cc
Resp.
Rt. Carotid
Dog:-7.5 Kilos: Evipan 1 gm. by Stomach
No tremors or Convulsions. +230 mg. by vein.
Base Line - 4 Seconds.

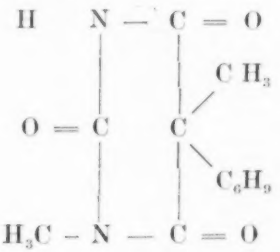
Fig. 4.—Metrazol following evipal.

stopped, will the ether tension in the patient's blood and medulla fall low enough, within reasonable limits of time, to allow the metrazol to exert a therapeutically useful effect on the center? For the present, these questions must go unanswered, but I suspect that the ultimate answer will be "yes" in both instances.

Here I should perhaps emphasize that, in the light of comparison with experiments described below, ether appears to possess a specific depressant

action on the respiratory center. For metrazol tends to produce tremors and convulsions, rather than respiratory stimulation, in dogs that are fully anesthetized with ether. And this is not simply a matter of the size of the dose, for a dose of metrazol of sufficient size to produce in an etherized dog convulsions only, with no efficient stimulation of the respiratory center, will produce a marked specific stimulation of the respiratory center with no vestige of tremors or convulsions in a dog which has been comparably anesthetized with evipal (see Fig. 3).

The contrast between the actions of evipal (evipan) and metrazol on the respiratory center are further illustrated in Fig. 4. This tracing was taken from a small dog (7.5 kilos) yet 3 c.c. (4.5 grains) of metrazol produced profound, vigorous, and rapid respiration by a direct stimulation of the respiratory center, while no tremors or convulsions whatever could be observed. The slow and rather weak character of the respiration previous to the injection of the metrazol shows clearly that the animal was deeply anesthetized by the evipal (evipan). The action of evipal (the new name for evipan) is of shorter duration than that of most of the older hypnotic drugs. Hence in this experiment a solution of evipal was kept at hand and small additional doses were injected from a buret intravenously from time to time in order to hold the anesthesia at any depth desired. An original dose of one gram dissolved in water and given by stomach tube produced a deep, quiet anesthesia in this dog (7.5 kilos) in five or six minutes. This drug may be very useful for experimental purposes, for by occasional small intravenous injections of later doses of the drug, the anesthesia can be kept at almost any desired level, and the specific counteracting effect of metrazol on the respiratory center can be used to revive the animal and arouse it almost to a state of consciousness. This metrazol stimulation, however, can be at once counteracted by an additional dose of evipal. This counteracting effect of the two drugs may be repeated many times during the course of a long experiment (see Fig. 5, nembutal). Evipal is N-methyl-cyclohexenyl-methyl-malonyl-urea having the following formula:



The sodium salt is readily soluble in water but is unstable and the powder should be freshly dissolved in water immediately before injection (by stomach tube, intravenously or intraperitoneally).

The striking antagonistic actions of metrazol and nembutal are well shown in Fig. 5. This animal was deeply anesthetized with nembutal and at the beginning of the tracing 8 c.c. of metrazol were injected by femoral vein. The

respiratory center was promptly stimulated but following this 2 gr. of nembutal were injected and this immediately depressed the respiration. There was a sharp temporary fall in blood pressure following each of these injections.

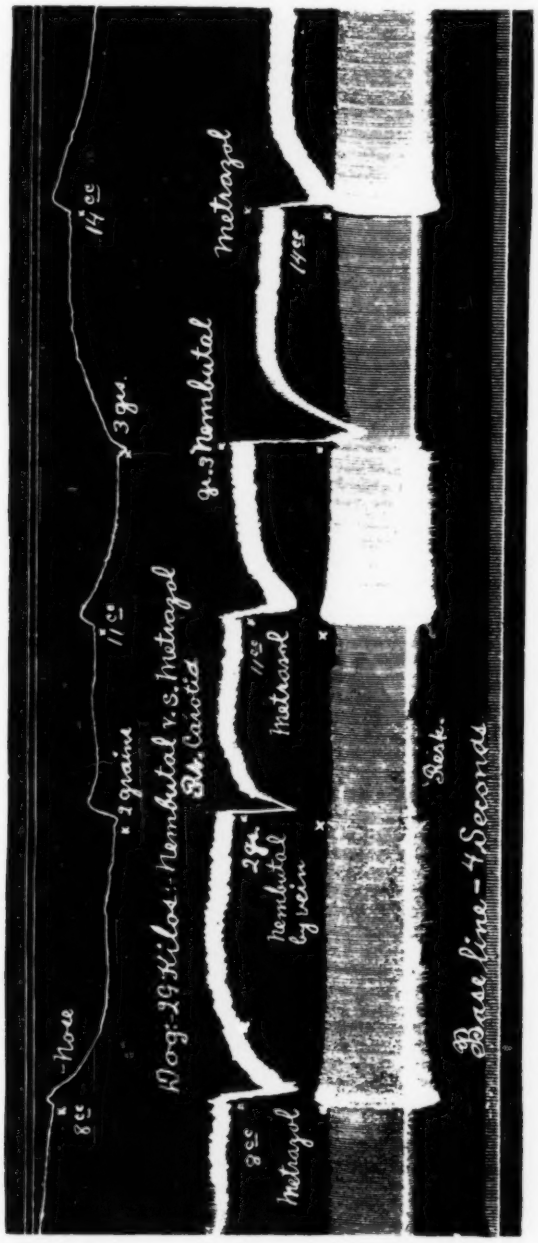


Fig. 5.—Five injections showing the alternate opposing actions of metrazol and nembutal.

This type of vascular reaction is common to a great many substances and its cause (or causes) is not well understood. It may be due to the sudden application to the endocardium of a rather strong solution of the drug (Abel), or to a similar sudden application of the solution to the medullary or lower

centers, or possibly to the lining of the arterioles, etc. These temporary blood pressure falls are of no especial significance, but there is one point which

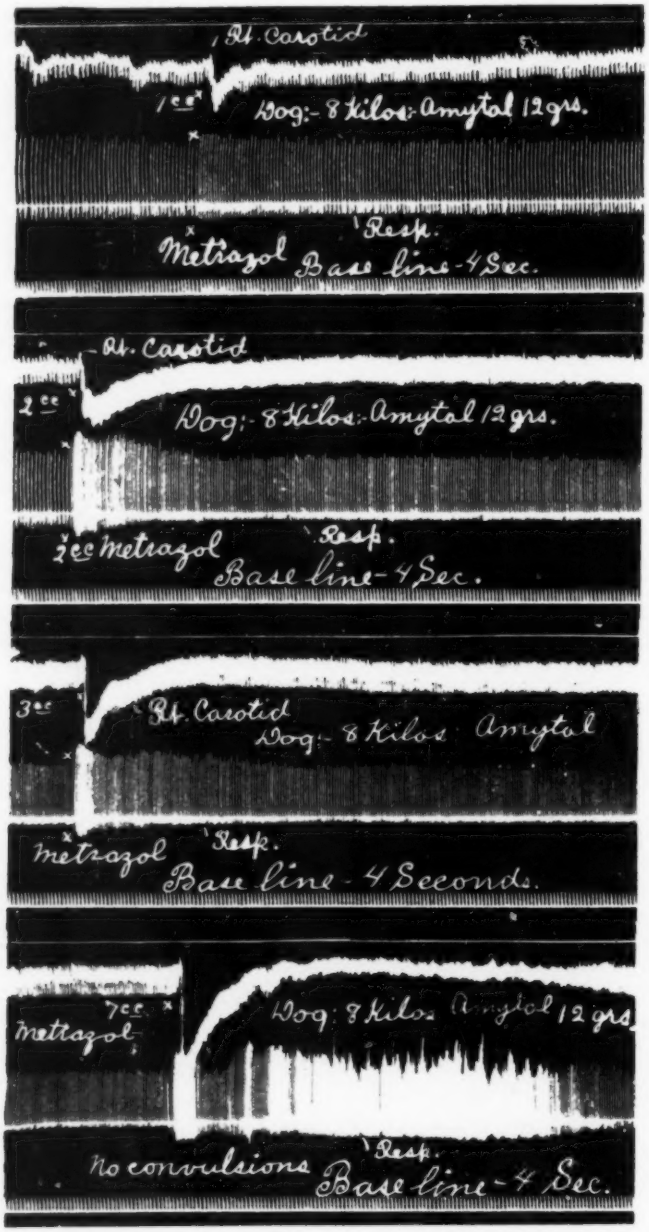


Fig. 6.—Four sections from the same experiment mounted consecutively from top to bottom and showing the effect of increasing dosage of metrazol on a dog anesthetized with amytal.

should be kept in mind in experiments of this type, viz., that a sudden, sharp fall in blood pressure may produce sufficient anemia, and hence asphyxia, in the medulla to act as a stimulation on the respiratory center. Since the first

fall in pressure (metrazol) in this tracing was accompanied by a stimulation of the respiration, and the second fall (nembutal) by a depression of the respiration it seems obvious that the blood pressure changes had little or no

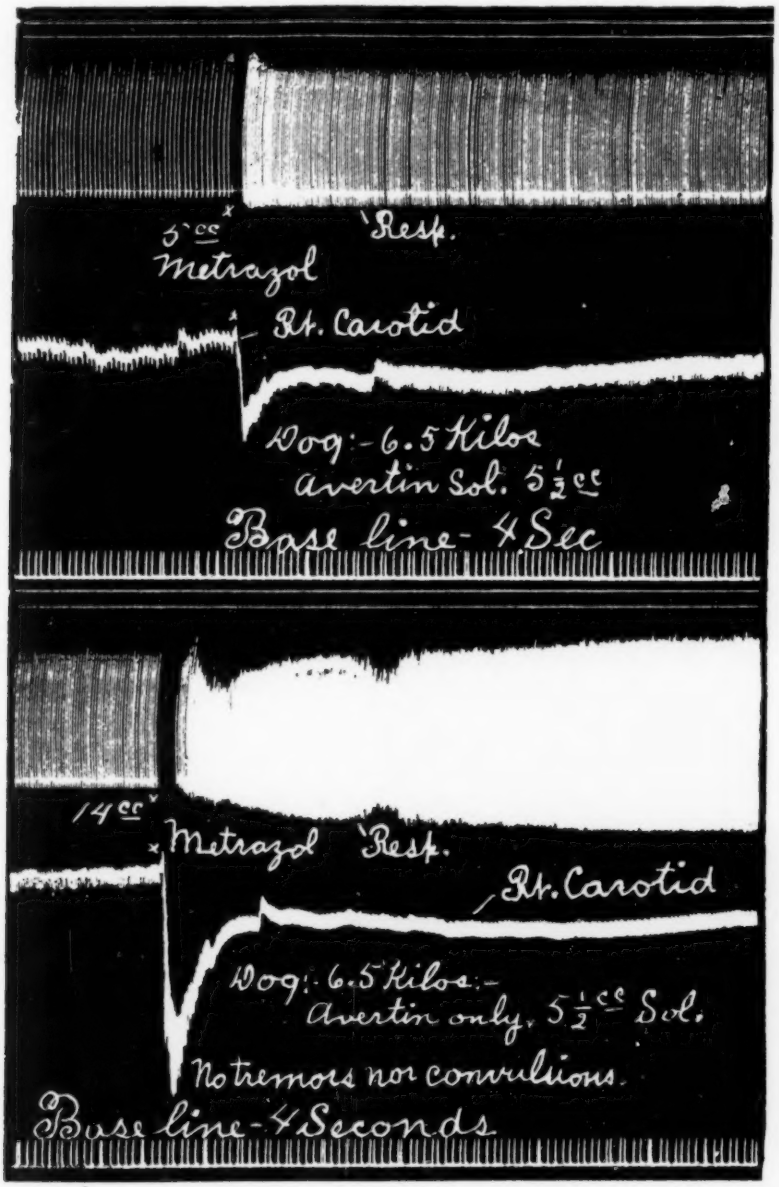


Fig. 7.—Two sections from the same experiment mounted consecutively from top to bottom and showing the effect of increasing dosage of metrazol on a dog anesthetized with avertin.

effect on the respiration. The three subsequent injections of metrazol, nembutal, and metrazol show the counteracting effects of these two drugs on the respiratory center.

Attention should be called to the matter of dosage in these experiments. Dogs are much less sensitive to these drugs than are human beings, so that while a dose of metrazol which is apparently out of all proportion to the amount which could be given to a patient may be injected into a dog, still

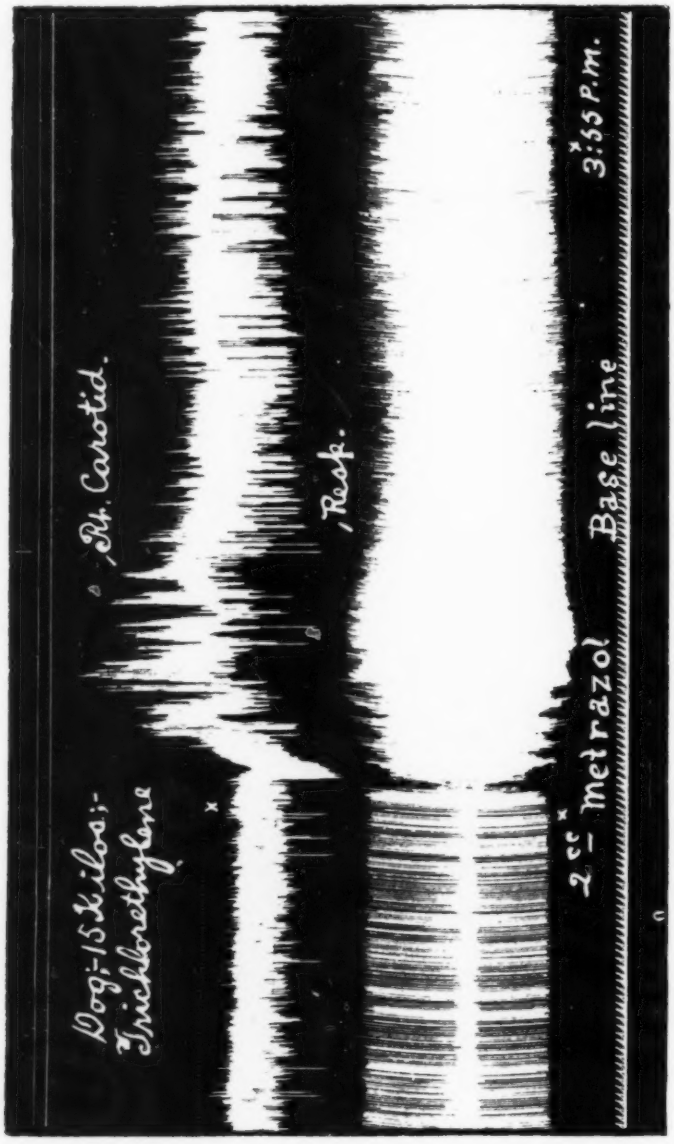


Fig. 8.—Tracing showing the effects of metrazol on a dog anesthetized with trichlorethylene.

it should also be noted that a correspondingly large increase in the size of the dose of the hypnotic has been required to produce the anesthesia. And the specific opposing actions of such drugs as evipal and metrazol on the respiratory center seems to be so definite and clear cut that, after the first injection of each drug (to test the individual sensitivity of the animal), one can thereafter predict with a high degree of accuracy the exact size of the

dose of each drug which will be needed just to counteract the effects of the other. It must be held in mind, of course, that, within the limits of tolerance, the animal becomes progressively less and less susceptible to each succeeding dose of a given drug, so that the amount injected each time must be progressively increased to make up for this loss in susceptibility.

A very similar counteracting action on the respiratory center is manifested by amytal and metrazol (Fig. 6) and by avertin and metrazol (Fig. 7).

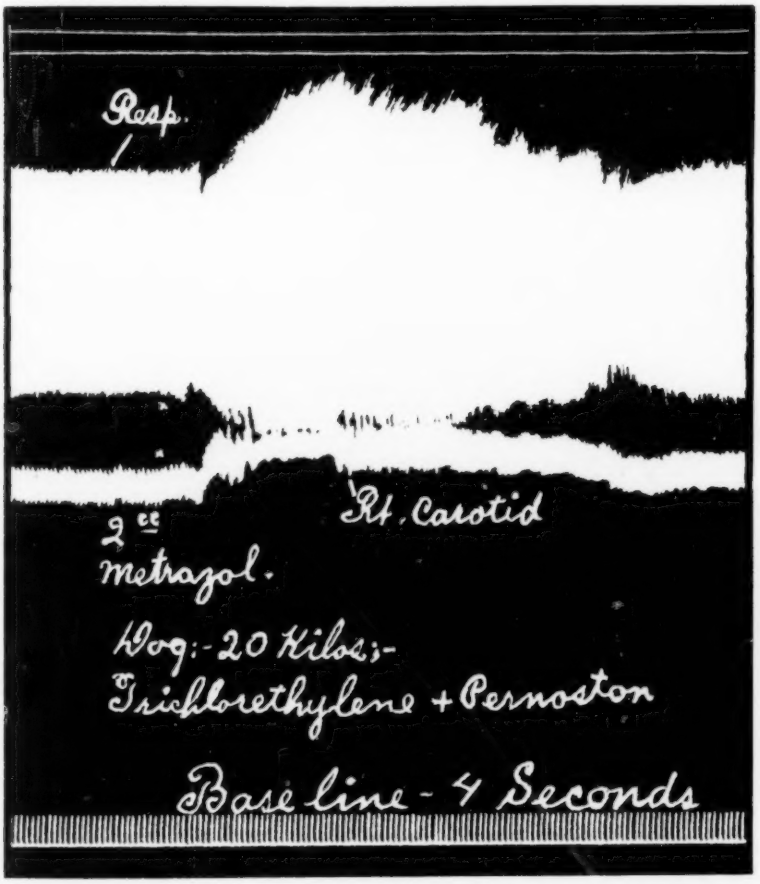


Fig. 9.—Tracing showing the effects of metrazol on a dog anesthetized with trichlorethylene and pernocton. Note the rise in blood pressure due mainly to contraction of the splanchnic blood vessels.

This shows that these drugs of widely differing chemical structure still differ quite markedly from ether in the action which they manifest on the respiratory center and on the other centers of the medulla and the spinal cord. For huge doses of metrazol are unlikely to produce convulsions in animals deeply anesthetized by amytal or avertin (as would happen under ether) but on the other hand metrazol markedly and specifically stimulates the respiratory center in animals anesthetized by amytal or by avertin, but has little or no specific respiratory stimulating action in animals anesthetized by ether.

It is extremely probable that all drugs which stimulate the respiratory center (or any other part of the central nervous system) could be shown to manifest some such varying reactions when administered to animals anesthetized by a series of different chemical substances. And it is probable that these variations might often be of therapeutic importance. For this particular series of experiments metrazol has simply served as a convenient agent to reveal and emphasize a few facts which are ordinarily obscured by the soothing influence of a deep sleep.

Fig. 8 shows the action of metrazol on a dog anesthetized with trichlorethylene. The type of anesthesia produced by trichlorethylene is different from those produced by either the hypnotics or ether. Yet it can be seen from this tracing that metrazol stimulates the respiratory center in dogs anesthetized with trichlorethylene. It appears that the anesthesia produced by this drug resembles in varying degrees the anesthesias produced by both ether and the hypnotics. For here metrazol stimulates both the respiratory center and, to a less extent, the reflexes of the cord and perhaps of the medulla. The metrazol tends to produce tremors and mild convulsive movements, as well as a marked increase in respiratory rate.

Fig. 9 shows the influence of metrazol on a dog anesthetized by both trichlorethylene and pernocton. Here the respiration is again markedly stimulated but there is considerably less tendency for the development of tremors and convulsive twitching. Apparently the (cord and medulla) stimulating action of the metrazol has been efficiently counteracted by the pernocton. And here a small dose (2 c.c.) of metrazol produced a marked respiratory stimulation in a large (20 kilos) dog.

Experimentally it seems that unanesthetized dogs are very much more sensitive to metrazol than is the case when the animal is under the influence of any of the central nervous depressing drugs whatever. And I suspect that generalized tremors or convulsions, without specific respiratory stimulation, are rather more likely to occur if no anesthetic substance is used. Whether similar reactions would occur in man in the absence of a central nervous depressant I cannot say. But this point might be of considerable interest clinically in the treatment of shock from trauma, drowning, poisoning, etc. From the varying reports of the therapeutic usefulness of metrazol in the literature, ranging from excellent to questionable, I suspect that the drug is very efficient in the proper cases, but inefficient in others.

CONCLUSIONS

1. No two general anesthetics, or hypnotics, possess the same action on the central nervous system. When the anesthesia produced by each of two substances appears to be exactly the same, a marked difference may frequently be shown if a substance having a specific stimulating action on some particular part or center of the central nervous system be injected to counteract the anesthesia.

2. In a dog anesthetized with ether, metrazol produces marked generalized tremors or convulsions, but with little or no specific stimulation of the respiratory center.

3. In a dog deeply anesthetized with evipal (and many other of the more recent hypnotics), metrazol produces marked specific stimulation of the respiratory center but without any symptoms of tremors or convulsions.

4. In a dog anesthetized with trichlorethylene, metrazol produces a combination of these reactions. The respiratory center is stimulated and some tremors and convulsive twitchings are produced.

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THE MECHANISM OF THE BLOOD CHANGES DURING THE TREATMENT OF SECONDARY AND PERNICIOUS ANEMIA*

C. A. Elvehjem, Ph.D., and M. O. Schultze, M.S., Madison, Wis.

THE fact that reticulocyte responses very similar in nature have been observed during the treatment of both pernicious and secondary anemias introduces an interesting question concerning the mechanism of these blood changes. Minot and Heath[1] have presented examples of the course taken by the reticulocytes in response to potent liver preparations in pernicious anemia and in response to maximal amounts of iron in anemia chiefly due to chronic blood loss. In general, the responses are very similar except that those in the case of secondary anemia are more prolonged.

Schultze and Elvehjem[2] have recently demonstrated that a typical reticulocyte response in anemic rats can be obtained only when adequate amounts of both iron and copper are fed, and they suggest that the initiation of reticulocytosis is dependent upon newly formed hemoglobin and that the subsequent decrease in the number of these cells is influenced by the amount of hemoglobin present in the blood. When suboptimal amounts of either iron or copper were fed, a much prolonged response occurred. In the light of these facts, the results

*From the Department of Agricultural Chemistry, University of Wisconsin.
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recorded by Minot and Heath[1] in secondary anemias may have been affected by an insufficient supply of copper.

A true explanation of the rapid changes in the number of reticulocytes in the blood during the treatment of anemias will depend to a large extent upon histologic studies of the hematopoietic organs which produce these cells—mainly the bone marrow. This, of course, is rather difficult in human patients. The histologic changes need to be studied daily in order that they may be correlated with the blood changes. In the case of secondary anemia such studies can now be made with rats. In the meantime we wish to suggest the following explanation of the changes observed in the blood during the treatment of pernicious and secondary anemias.

In order to simplify the discussion, the deficiency in pernicious anemia will be considered as a lack of ability to form red cells and the deficiency in secondary anemia a lack of adequate hemoglobin-building power. The bone marrow will be considered as the center for red cell formation and the liver the centralized organ for hemoglobin production. There may be some criticism of the latter assumption but this will not affect the discussion. An adequate supply of the P.A. factor will be considered positive treatment for pernicious anemia and an adequate supply of iron and copper positive treatment for secondary hypochromic anemia. No attempt will be made to discuss the source or methods of supplying these materials or the more complicated forms of anemia which are encountered.

A condensed outline of the changes taking place in the liver, bone marrow, and blood during the development and treatment of secondary and per-

TABLE I

CHANGES OCCURRING DURING THE DEVELOPMENT AND TREATMENT OF SECONDARY AND PERNICIOUS ANEMIAS

STAGE	TISSUE	SECONDARY	PERNICIOUS
Development	Liver	Deficiency of iron and/or copper. No Hb. formation	Sufficient iron and copper. Hemosiderosis
	Bone marrow	P. A. factor available	Deficiency of P. A. factor. Fragile cells produced. Exhaustion
	Blood	Decrease in total Hb. Decrease in Hb. per cell Decrease in no. of red cells	Decrease in no. of red cells. Increase in Hb. per cell. Decrease in Hb.
Treatment with suboptimal amounts of iron and/or copper (secondary anemia) or small amounts of P.A. factor (pernicious anemia)	Liver	Some Hb. formation	
	Bone marrow	Hb. stimulates cell formation	Slow cell formation
	Blood	Increase in reticulocytes. Insufficient Hb. to form normal cells. Hb. and cells low. Reticulocytes high	Prolonged reticulocyte response. Small increase in cells and hemoglobin
Treatment with adequate amounts of iron and copper (secondary anemia) or optimum amounts of P.A. factor (pernicious anemia)	Liver	Rapid Hb. formation	Hemoglobin formation if sufficient Fe and Cu are present
	Bone marrow	Rapid cell formation	Rapid cell formation
	Blood	Typical reticulocyte response. Increase in Hb. and red cells	Typical reticulocyte response. Increase in cells and Hb.

nicious anemias is given in Table I. In secondary anemia there is a deficiency of iron and/or copper due either to a deficient supply from the food or to a too rapid utilization of the stores of these elements in the body. When these elements are no longer available, the liver is unable to build sufficient hemoglobin, and consequently, there is a decrease in the total hemoglobin of the blood. For a time the cells continue to be produced, which fact causes a decrease in the amount of hemoglobin per unit of cell. The new cells which are formed cannot be supplied with hemoglobin and they are destroyed by the reticuloendothelial system. The bone marrow soon gives up its attempt to manufacture cells and a condition of aplasia develops. McGowan[3] has observed an aplastic condition in the bone marrow of severely anemic pigs. The ordinary marrow elements were present, but there was an absence of signs of activity and the cells were greatly reduced in numbers.

When suboptimal amounts of iron and/or copper are supplied in this type of anemia, a small amount of hemoglobin is produced immediately. The presence of this newly formed hemoglobin (or a possible precursor) stimulates the bone marrow to rapid cell formation and causes a very definite increase in the number of reticulated cells in the circulating blood. The quantity of hemoglobin formed in this case is limited and the new cells do not receive a normal supply of pigment. These cells are destroyed and the stimulus for new cell formation continues. Under such conditions a prolonged reticulocyte response similar to that described by Schultze and Elvehjem[2] is obtained.

If adequate amounts of iron and copper are available, the picture is quite different. Rapid hemoglobin formation takes place in the liver. The newly formed hemoglobin stimulates cell formation and the bone marrow pours the reticulocytes into the blood. Now the new cells are supplied with sufficient hemoglobin; they mature into normal red cells, and the impetus for further cell formation is reduced. In a short time the hemoglobin and red cells approximate normal values and the number of reticulocytes decreases because only a small number of new cells need to be formed. The greatest reticulocyte response occurs when the hemoglobin and red cells are the lowest because there is then a greater requirement for new cells. Cell formation is not complicated by an insufficiency of stroma building material in true secondary anemia because there is plenty of the P.A. factor available.

In pernicious anemia the condition is quite different, here hemoglobin can be formed, but the bone marrow is unable to produce cells due to the absence of the P.A. factor. The cells which are formed are fragile and are destroyed immediately. The stimulus for cell production is present and the marrow attempts to produce cells until it becomes completely exhausted. Since hemoglobin can be formed, the cells present in the blood carry an increased amount of pigment. When the cells are destroyed, the hemoglobin cannot be carried by the blood and hemosiderosis takes place due to the accumulation of the iron set free from the destroyed cells.

When small amounts of the P.A. factor are supplied, the bone marrow produces new cells slowly, the reticulocyte response will be small and prolonged, and the hemoglobin and red cells increase only slightly. In the presence of optimum amounts the new cells are formed rapidly and a decided increase in the reticulocytes is observed. There is sufficient hemoglobin to supply

the new cells and the mature cells are readily formed. The impetus for cell formation decreases and a corresponding decrease in reticulocytes follows. The reticulocyte response may be prolonged if there is not sufficient iron and copper present for rapid hemoglobin formation.

This explanation is very brief and general in character, but we hope it will aid in formulating certain fundamental principles necessary for a more complete understanding of anemia.

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THE EFFECT OF ULTRAVIOLET LIGHT ON SOME SYMPATHOMIMETIC SUBSTANCES*

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THE action of ultraviolet light on drugs presents an insight into the changes that occur in such drugs in everyday experience when they are exposed to ordinary daylight. The photochemical action of light is chiefly due to the ultraviolet region; consequently, exposure to the rays of a quartz mercury arc lamp for a short time corresponds to a long exposure to daylight. This investigation is a continuation of work previously published.[1]

Relatively little work has been done on this particular problem. Savopol,[15, 16, 17] Verda, Kneer, and Burge,[20] Kneer, Orth, Verda and Burge,[6] and Ito and Terata[5] have reported on the effect of ultraviolet irradiation of solutions of epinephrine. The action of these rays on other drugs has been reported by a few investigators: Pacini and McGuigan,[11] Wakeham and Tracy,[21] Higgins, Ewing, and McGuigan,[3] and Gant[2] on nicotine; Macht[9] on digitalis. In the majority of instances, destruction of the drug is found. As was shown in our previous paper,[1] this may not always be the case.

This study is limited to three sympathomimetic drugs—epinephrine (adrenalin—Parke, Davis and Company), synephrin (Frederick Stearns and Company, N. N. R., 1931), and p-hydroxyphenyl-l-amino-2-propanol-l (Sharp and Dohme).

METHODS

Ultraviolet Irradiation.—From 10 to 30 c.c. of 0.01 per cent epinephrine, 0.1 per cent synephrin, or 0.1 per cent p-hydroxyphenylpropanolamine solu-

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tion was exposed either in open Petri dishes (9.8 cm. diameter) or clear quartz covered dishes (6.8 cm. diameter) at a distance of 20 cm. from a Cooper-Hewitt mercury arc lamp (35 ergs per mm.^{2}) for from ½ minute to 20 hours at a temperature of 20° C. when water cooled or a temperature of 55° C. when air cooled.

Boiling With H_2O_2.—Ten cubic centimeters of the above solutions were boiled moderately in a 50 c.c. beaker over a free flame from 1 to 35 minutes with varying amounts of H_2O_2 added.

Irradiation With H_2O_2.—Solutions were irradiated as in the first step, but with the addition of H_2O_2 before exposure to the rays.

Biologic Assay.—Method 1: Change in blood pressure following intravenous injection in dogs was determined according to the method described in U.S.P. X, p. 212, for assay of liquor epinephrinae hydrochloridi. Anesthesia—nembutal (Abbott) 20 mg. per kg. intraperitoneally preceded by 3 mg. per kg. morphine sulphate subcutaneously; in a few cases—ether, preceded by morphine.

Method 2: The hyperglycemic and glycosuric actions following intraperitoneal injections of the drugs in rabbits were determined. Samples of blood and urine were taken from unanesthetized animals (starved 24 hr. or more) before the injection and 1 or 2 hours after injection of the drug. Blood sugar was determined by the Folin-Wu method and urinary sugar with Benedict's qualitative reagent.

Method 3: The inhibitory effect of the drugs on the isolated rat uterus using the usual means of recording contractions in aerated Locke-Ringer solution.

Method 4: The effect of intravenous injection of the drugs in relieving or preventing bronchial constriction induced by histamine in guinea pigs was studied according to the method of Koessler and Lewis.[7]

RESULTS

Epinephrine.—Irradiation of a 1:10,000 solution of epinephrine HCl resulted in a loss of activity as shown in Table I with short intervals of exposure, and in Table II and Fig. 1, with long intervals. These results

TABLE I

Dog—Weight, 12 kg. Vagi atropinized
Date—Sept. 13, 1932. Anesthesia—morphine, nembutal
Injections: 0.2 c.c. epinephrine HCl 0.01 per cent
Irradiation: 20 c.c. 0.01 per cent solution in quartz dish at 20 cm., air cooled; 2 c.c. samples removed at each time interval

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0.0	138	204	66	
2	0.5	136	192	56	
3	1.0	140	193	53	
4	1.5	150	196	46	
5	2.0	152	190	38	
6	3.0	150	182	32	
7	5.0	144	173	29	

confirm previous publications of our own and of other investigators[1, 20, 6] and fail to show any increase as reported by Ito and Terata. The difference in time necessary to produce these changes is due to the manner of irradia-

Fig. 1.

tion, the chief factors being distance from the arc, volume of solution, concentration of solution, and temperature. The predominance of depressor action in irradiated epinephrine solutions is seen in the 330-minute sample (Table II). This depressor action is eventually lost with continued irradia-

tion. A 0.01 per cent solution of epinephrine alkaloid loses its activity by irradiation as does the HCl salt, the loss being even more rapid in the presence of Na_2CO_3 (Table III). Irradiation of epinephrine HCl solution with the addition of H_2O_2 markedly increases the rate of destruction (Table IV). Boiling the drug with H_2O_2 also hastens its destruction (Table V).

TABLE II

Dog—Weight, 12 kg. Vagi atropinized
Date—Sept. 15, 1932. Anesthesia—morphine, nembutal
Injections: 0.3 c.c. epinephrine HCl 0.01 per cent
Irradiation: 20 c.c. 0.01 per cent solution in quartz dish at 20 cm., water cooled; 2 c.c. removed at each time interval

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	138	192	54	
2	60	136	168	32	
3	120	137	166	29	
4	180	141	156	15	
5	240	141	148	7	
6	300	144	148	4	
7	330	147	141	−6	
8	0	141	190	49	

TABLE III

Dog—Weight, 9 kg. Vagi atropinized
Date—June 16, 1931. Anesthesia—morphine, nembutal
Injections: 1 c.c. 0.01 per cent epinephrine alkaloid (P. D. and Co.) (see remarks)
Irradiation: 10 c.c. 0.01 per cent solution in Petri dish at 20 cm., air cooled, some with acid or alkali added (remarks)

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	48	145	97	
2	60	56	62	6	
3	60	60	69	9	Irradiated with HCl
4	60	62	63	1	Irradiated with Na_2CO_3

TABLE IV

Dog—Weight, 9 kg. Vagi atropinized
Date—July 19, 1933. Anesthesia—morphine, nembutal, ether
Injections: 0.2 c.c. of 0.01 per cent solution epinephrine HCl, except No. 5
Irradiation: 10 c.c. 0.01 per cent solution in quartz dishes at 20 cm., water cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	108	159	51	Control
2	35	110	149	39	No peroxide added
3	35	111	123	12	With 2 drops 2 per cent peroxide
4	35	109	111	2	With 5 drops 2 per cent peroxide
5	0	108	108	0	(Control peroxide—5 drops 2 per cent peroxide in 10 c.c. of water)

These same changes in epinephrine solutions are shown in tests of glycosuric action in rabbits (Tables VI and VII), on the relief of histamine bronchiospasms in guinea pigs (Table VIII), and on the isolated rat uterine strip (Table IX).

TABLE V

Dog—Weight, 11 kg. Vagi cut
Date—Sept. 14, 1931. Anesthesia—morphine, nembutal
Injections: 0.5 c.c. epinephrine HCl 0.01 per cent
Oxidation: 10 c.c. 0.01 per cent solution boiled 10 minutes with 1 per cent H_2O_2 (remarks)

NO.	MINUTES BOILED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	10	114	198	84	No peroxide
2	10	118	193	75	With 1 drop H_2O_2
3	10	113	172	59	With 2 drops H_2O_2
4	10	113	146	33	With 5 drops H_2O_2

TABLE VI

Rabbits—About 2.0 kg. wt.
Date—Aug. 14, 1931
Injection: Epinephrine HCl, 0.01 per cent, intraperitoneally
Irradiation: 30 c.c. samples 0.01 per cent solution in Petri dish at 20 cm., air cooled

RABBIT NO.	MINUTES IRRADIATED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA
			NORMAL MG. PER 100 C.C.	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	1.0	121	3	235	++
2	60	1.5	129	3	125	–
3	60	3.0	118	3	115	–
4	0	1.0	167	1	290	++
5	60	3.0	118	1	267	+
6	180	10.0	139	1	180	–
7	0	0.5	110	1	258	++

TABLE VII

Rabbits—About 2 kg. in weight
Date—Sept. 6, 1931
Injections: Epinephrine HCl, 0.01 per cent, intraperitoneally
Oxidation: 20 c.c. 0.01 per cent solution boiled with 2 drops 1 per cent H_2O_2

RABBIT	MINUTES BOILED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA
			NORMAL MG. PER 100 C.C.	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	0.5	107	1	236	+
2	1	1.5	112	1	196	–
3	1	3.0	104	1	222	+
4	10	8.0	115	1	174	–

TABLE VIII

EFFECT OF EPINEPHRINE HCL ON RECOVERY FROM BRONCHIOLE CONSTRICTION INDUCED BY 0.06 MG. HISTAMINE PICRATE*

Guinea pig—Weight 620 gm.
Date—Aug. 2, 1933. Anesthesia—pithed
Injections: Histamine picrate, 1 c.c. of 0.006 per cent solution intravenously. Epinephrine HCl, 1 c.c. 0.005 per cent solution intravenously
Irradiation: 10 c.c. 0.01 per cent solution irradiated 90 minutes in quartz dish at 20 cm., air cooled

MINUTES AFTER HISTAMINE	0	1	2	3	4	5
Control	1	4	8	20	34	40
Irradiated epinephrine HCl at†	1	4	12†	23	32	39
Nonirradiated epinephrine HCl at†	1	3	8†	29	33	35

*Figures represent excursion of lever in millimeters.

Synephrin.—Solutions of synephrin salts when exposed to ultraviolet rays regularly exhibit an increase in action as was shown in our preliminary report.[1] A maximum point is reached, however, when further irradiation effects a breakdown of the active substance until it is practically destroyed (Fig. 2 and Table X). The increased activity may be from 10 to 20 times the

TABLE IX

Epinephrine on uterine strip of rat
Date—July 27, 1933
Injections: Specified dose of 0.01 per cent epinephrine HCl added to cup containing uterine strip in 50 c.c. Locke-Ringer solution
Irradiation: 10 c.c. 0.01 per cent epinephrine HCl irradiated 90 minutes in quartz dish at 20 cm., air cooled

DOSE	DRUG	CONTRACTIONS (MOVEMENTS OF LEVER IN MM.)	
		NORMAL	AFTER INJECTION
0.01 c.c.	Epinephrine HCl nonirradiated	90	50
0.02 c.c.	Epinephrine HCl nonirradiated	90	−10
0.02 c.c.	Epinephrine HCl irradiated	90	42

TABLE X

Dog—Weight, 5.8 kg. Vagi cut
Date—Feb. 10, 1933. Anesthesia—morphine, ether
Injections: 1.0 c.c. synephrin, HCl 0.1 per cent
Irradiation: 20 c.c. 0.1 per cent solution in quartz dish at 20 cm., water cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	51	58	7	
2	360	53	114	61	
3	720	33	54	21	
4	1080	35	39	4	
5	1200	39	40	1	
6	0	42	50	8	

TABLE XI

Dog—Weight, 9 kg. Vagi atropinized
Date—Sept. 11, 1931. Anesthesia—morphine, nembutal
Injections: 0.5 c.c. synephrin, HCl 0.1 per cent
Oxidation: 10 c.c. 0.1 per cent solution irradiated with 2 drops 1 per cent H_2O_2 in Petri dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	1	128	206	78	
2	4	130	270	140	
3	10	110	260	150	
4	20	102	256	154	
5	60	95	240	145	
6	120	100	233	133	

normal activity. Irradiation of the solution with the addition of a small amount of H_2O_2 markedly decreases the time necessary to produce these changes (Table XI), and simply boiling the solution with H_2O_2 effects a similar increase in activity (Table XII).

Irradiation of a solution of synephrin *base* for 60 minutes does not produce any increase in activity, while the addition of an acid makes the change

very evident (Tables XIII and XIV). The effect of varying the P_H of the synephrin solution is discussed later (under heading, "Chemistry"). These

Fig. 2.

hyperactive solutions maintain their activity for a relatively long time since we have found that solutions kept in the laboratory for six months at room temperature were as active as when freshly prepared.

TABLE XII

Dog—Weight, 9 kg. Vagi atropinized
Date—Sept. 11, 1931. Anesthesia—morphine, nembutal
Injections: 0.5 c.c. synephrin HCl 0.1 per cent
Oxidation: 10 c.c. samples 0.1 per cent synephrin HCl (except No. 4) boiled with 2 drops of 1 per cent H_2O_2

NO.	MINUTES BOILED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	100	110	10	
2	1	102	161	59	
3	4	104	187	83	
4	8	100	146	46	1 per cent synephrin HCl boiled, then diluted to 0.1 per cent before injection
5	10	99	226	127	
6	30	99	219	120	

TABLE XIII

Dog—Weight, 9 kg. Vagi atropinized
Date—Feb. 18, 1931. Anesthesia—morphine, ether
Injections: 0.5 c.c. of 0.1 per cent synephrin base or hydrochloride
Irradiation: 10 c.c. 0.1 per cent solution in Petri dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	150	159	9	Synephrin base
2	60	152	161	9	
3	0	153	163	10	Synephrin HCl
4	60	156	260	104	

TABLE XIV

Dog—Weight, 12 kg. Vagi atropinized
Date—Feb. 25, 1931. Anesthesia—morphine, nembutal, ether
Injections: 1.0 c.c. synephrin base 0.1 per cent (see remarks)
Irradiation: 10 c.c. of above solution in Petri dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	153	162	9	
2	60	155	225	70	Irradiated with citric acid
3	60	154	221	67	Irradiated with oxalic acid
4	60	155	232	77	Irradiated with succinic acid

TABLE XV

Rabbits—About 2.0 kg. in weight
Date—Aug. 24, 1931
Injections: Synephrin HCl 0.1 per cent intraperitoneally
Irradiation: 30 c.c. samples 0.1 per cent solution in Petri dish at 20 cm., air cooled

RABBIT	MINUTES IRRADIATED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA
			NORMAL (MG. PER 100 C.C.)	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	40	122	1	111	–
2	60	35	105	1	115	–
3	180	35	139	1	303	++
4	180	20	119	1	250	++
5	180	30	93	2	200	++
6	0	100	120	2	138	–
7	0	300	89	2	129	–

The increased effectiveness of irradiated solutions of synephrin salts is demonstrated in the marked action of relatively small doses in producing hyperglycemia and glycosuria (Table XV). A marked hyperglycemia and glycosuria has been produced in rabbits with as little as 8 mg. of irradiated drug. Boiling the drug with H_2O_2 increases this action also, as shown in Table XVI.

Irradiated synephrin solutions are effective in relieving histamine bronchiospasms of guinea pigs (Table XVII) or preventing these spasms by previous injection of synephrin (Table XVIII). The same dose of nonirradiated drug had no noticeable effect.

TABLE XVI

Rabbits—About 2 kg. in weight
Date—Sept. 30, 1931
Injection: Synephrin, HCl 0.1 per cent, intraperitoneally
Oxidation: 20 c.c. samples of 0.1 per cent solution boiled with 2 drops 1 per cent H_2O_2

RABBIT	MINUTES BOILED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA PRODUCED
			NORMAL (MG. PER 100 C.C.)	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	100	111	1	142	–
2	1	40	109	1	121	–
3	5	40	117	1	186	–
4	10	20	114	1	232	++

TABLE XVII

Guinea pig—Weight, 533 gm.
Date—July 14, 1933. Anesthesia—pithed
Injections: Histamine (Pfanstiehl) 2 c.c., 0.001 per cent solution, intravenously. Irradiated synephrin HCl 2 c.c., 0.1 per cent, intravenously
Irradiation: 10 c.c. synephrin HCl (0.1 per cent) in quartz dish at 20 cm., air cooled, irradiated 90 minutes

	EXCURSION OF LEVER (MM.)		
	I CONTROL	II CONTROL	III WITH SYNEPHRIN
Normal excursion	60	70	60
0.02 mg. histamine injected	--	--	--
After 1 minute	1	1	1
After 2 minutes	1	2	1
After 3 minutes	2	3	20*
After 4 minutes	6	3	30

*Injection of 2 mg. irradiated synephrin HCl here.

TABLE XVIII

RESPONSE OF BRONCHIOLES TO INJECTIONS OF 0.06 MG. HISTAMINE PICRATE AFTER PREVIOUS INJECTION OF SYNEPHRIN (EXCURSION OF LEVER—MM.)

Guinea pig—Weight, 620 gm.
Date—Aug. 2, 1933. Anesthesia—pithed
Injections: Histamine picrate (Nelson) 1 c.c. of 0.006 per cent solution, intravenously. Synephrin HCl, 1.2 c.c. of 0.1 per cent solution, intravenously
Irradiation: 10 c.c. 0.1 per cent synephrin HCl irradiated 90 minutes in quartz dish, air cooled, at 20 cm.

HISTAMINE	NO SYNEPHRIN		NONIRRADIATED SYNEPHRIN HCl		IRRADIATED SYNEPHRIN HCl	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
After 7 min.	45	1	45	1	50	42
After 11 min.	35	1	35	1	50	14
After 15 min.	30	1	35	1	50	3
After 20 min.	32	1	36	1	50	1

The isolated rat uterine strip shows the increased effectiveness of irradiated synephrin in inhibiting normal contractions (Table XIX).

Parahydroxyphenylpropanolamine.—This drug reacts to ultraviolet irradiation with an increased pressor action. More than ten times the activity of the nonirradiated drug is shown in Table XX.

TABLE XIX

Synephrin on uterine strip of rat
Date—Sept. 27, 1933
Injections: Specified dose of 0.1 per cent synephrin HCl added to cup containing uterine strip in 50 c.c. Locke-Ringer solution
Irradiation: 10 c.c. 0.1 per cent synephrin HCl irradiated 90 minutes in quartz dish at 20 cm., air cooled

DOSE	DRUG	CONTRACTIONS (MOVEMENT OF LEVER IN MM.)	
		NORMAL	AFTER INJECTION
	Uterine Strip No. 1		
0.2 c.c.	Synephrin HCl nonirradiated	+96	+82
0.2 c.c.	Synephrin HCl irradiated	+98	−10
1.0 c.c.	Synephrin HCl nonirradiated	+96	− 6
	Uterine Strip No. 2		
0.1 c.c.	Synephrin HCl nonirradiated	75	50
0.1 c.c.	Synephrin HCl irradiated	80	−25

TABLE XX

Dog—Weight, 15.4 kg. Vagi atropinized
Date—Apr. 9, 1933. Anesthesia—morphine, nembutal
Injections: 0.5 c.c. of p-hydroxyphenyl-1-amino-2-propanol-1 hydrochloride. Control solution 0.1 per cent—irradiated solutions 0.01 per cent
Irradiation: 20 c.c. 0.01 per cent solution in quartz dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	116	140	24	Control 0.1 per cent
2	15	118	152	34	0.01 per cent solutions
3	35	116	153	37	
4	60	114	142	28	
5	125	114	128	14	

OXIDATION PRODUCTS OF EPINEPHRINE

We found that heating the drug with hydrogen peroxide and treatment with ultraviolet light, effects similar pharmacodynamic changes in the drug. For this reason and because of the relative simplicity in using hydrogen peroxide, this method was chosen in preparing the drugs for the study of the oxidation products.

Procedure: Five-tenths gram of pure epinephrine base was oxidized by refluxing with 100 c.c. of neutralized 5 per cent H_2O_2 for fifteen minutes. The solution developed a reddish brown color.

Tests for Oxidation Products.—1. Unchanged Epinephrine: The solution was cooled at once in ice water and excess oxygen removed by evacuation (in a flask at 1/150 atmosphere). The solution was first made acid with H_2SO_4 and distilled, then made alkaline with NaOH and distilled. The residue in the distilling flask when treated with phosphomolybdotungstic acid gave

an amethyst color, indicating the presence of epinephrine or catechol, both of which give this color with this reagent.

When $FeCl_3$ solution was added to the residue, a greenish blue coloration changing to red was produced, again indicating the presence of epinephrine or catechol.

Mitchell's reagent (0.2 per cent $FeSO_4$ + 1 per cent $KNaC_4H_4O_6$) gives with epinephrine a purple color and with catechol a pale blue. When this reagent was added to the residue, a pale blue color, changing to a purple, was observed.

NH_4OH with epinephrine produces a yellow color changing to orange and to pink; with catechol, it gives a green color, which fades rapidly. This reagent with the residue gave first a green color changing to orange and to pink and faded out. NH_4OH with Mitchell's reagent gives for both compounds a reddish violet changing to red. The same colors were obtained with the residue.

These tests indicate that the oxidized material still contained some unchanged epinephrine. This fact was further proved by blood pressure tests on a dog.

2. Catechol: Tests on the residue indicated the presence of catechol. The acid distillate when tested with phosphomolybdotungstic acid reagent gave a violet color; with ferric chloride solution a greenish blue; and with Mitchell's reagent a pale blue. Since epinephrine would not distill over, these tests confirm the presence of catechol. Millon's reagent with the distillate gave a negative test for the phenol group.

3. Ethyl Alcohol: When 5 c.c. of the acid distillate and 5 c.c. H_2SO_4 were titrated with $K_2Cr_2O_7$ (19 gm./liter), about 2 c.c. was required (Nicloux method). During the oxidation, the odor of acetaldehyde was perceptible, indicating the presence of an alcohol. When a few cubic centimeters of the distillate was warmed with a few drops of benzoyl chloride and NaOH, the odor of ethyl benzoate was observed, thus proving the presence of ethyl alcohol.

4. Methyl Alcohol: This test was negative by the U.S.P. (X) method.

5. Acetic Acid: The distillate was acid to litmus and, when heated, had the characteristic odor of acetic acid. When treated with a small amount of valerianic acid and $FeCl_3$, it gave a reddish violet color, indicating acetic acid.

6. Formic Acid: No formic acid was found.

7. Aldehyde: No test for aldehyde was obtained on the above distillate, probably because the oxidation was carried too far. To check this, a 0.1 per cent solution of epinephrine base was oxidized in the cold for a few minutes with H_2O_2, evacuated a few minutes, and then distilled. The first few cubic centimeters of distillate gave a positive reaction with Schiff's reagent (fuchsin sulphurous acid) and only a yellow color with morphine and sulphuric acid, thus proving the absence of formaldehyde and the presence of acetaldehyde.

8. Methylamine: The alkaline distillate had an ammoniacal fishlike odor, characteristic of methylamine. Rimini's test (1 c.c. acetone and 1 drop dilute

sodium nitroprusside) for an aliphatic amine was strongly positive, thus indicating the presence of methylamine.

From a study of these oxidation products, it appears that epinephrine, when oxidized, splits off the side chain, leaving the catechol nucleus. From the side chain, we first obtain ethyl alcohol and methylamine. Further oxidation of the alcohol yields acetaldehyde which in turn is oxidized to acetic acid. Catechol is the only decomposition product which has any appreciable effect on blood pressure and then very weak as compared to epinephrine.

The identification of these oxidation products is apparently sufficient evidence why epinephrine loses its pressor action when oxidized.

OXIDIZED SYNEPHRIN (CHEMICAL)

Five cubic centimeters of 10 per cent synephrin hydrochloride was boiled on a hot plate with 1 drop of 30 per cent superoxol until a deep red color developed—indicating the point when approximately maximum activity is reached.

Since synephrin base precipitates with NH_4OH in a 10 per cent solution, it was thought possible that the oxidized product might be separated in the same manner. Accordingly, the oxidized solution was cooled and 4 drops of concentrated NH_4OH added. The resulting precipitate was filtered on a suction filter, washed, and dried in a vacuum desiccator over H_2SO_4.

A. Precipitate tests:

1. Melting point of precipitate—170 to 171° C.
   Melting point of original synephrin base—173° C.
2. On blood pressure of dog:
   A 0.1 per cent solution of the precipitate had the same effect on blood pressure as the same concentration of synephrin base.
3. Chemical tests:
   a. Solution of the precipitate had a pale pink brown color.
   b. Test with Mitchell's reagent ($FeSO_4$ + Rochelle salt) shows no epinephrine or catechol.
   c. With $FeCl_3$—no catechol.
   d. With phosphomolybdotungstic acid—same color as with synephrin base.
   e. With phosphotungstic acid reagent + Na_2CO_3 (N.N.R.)—blue color a little darker than same amount of synephrin base—but not nearly as dark as epinephrine.

B. Test on filtrate:

On blood pressure of dog: Very slight activity which was not more than one-tenth of the activity of the original oxidized solution, but approximately the same as that produced by the same dose of synephrin base.

From the results of these tests, it appears that the precipitate obtained by alkalinization of the oxidized synephrin solution is only the unchanged

synephrin base. Furthermore, since the filtrate was not hyperactive, the active material seems to have been lost when the solution was alkalinized with NH_4OH. Combining the precipitate and filtrate and injecting into a dog gave no more rise in blood pressure than a simple additive effect of the two solutions.

These tests show only that the hyperactive oxidation product of synephrin is not precipitated by NH_4OH and is apparently rendered inactive by this reagent.

THE EFFECT OF HYDROGEN ION CONCENTRATION IN IRRADIATION OF SYNEPHRIN BASE

In earlier experiments, it was found that irradiation of synephrin base did not change its pressor activity whereas the hydrochloride, tartrate, citrate, oxalate, or succinate gave a marked change. As a further study of the effects of ultraviolet light on this drug, samples of 0.1 per cent synephrin base were made up and adjusted to a range of P_H values by the addition of N/20 HCl. A La Motte colorimetric outfit was used to determine the P_H values, all buffer solutions being freshly prepared. The value of the 0.1 per cent synephrin base solution was found to be P_H 9.3; N/20 HCl was added as indicated in Table XXI to make up solutions of P_H 9.0, 8.0, 7.0, 6.0, 5.0, 4.0, and 3.0.

TABLE XXI

		c.c. N/20 HCl	P_H
15 c.c. 0.1 per cent synephrin base	+	0	9.3
15 c.c. 0.1 per cent synephrin base	+	0.3	9.0
15 c.c. 0.1 per cent synephrin base	+	1.3	8.0
15 c.c. 0.1 per cent synephrin base	+	1.65	7.0
15 c.c. 0.1 per cent synephrin base	+	1.73	6.0
15 c.c. 0.1 per cent synephrin base	+	1.77	5.0
15 c.c. 0.1 per cent synephrin base	+	1.81	4.0
15 c.c. 0.1 per cent synephrin base	+	2.15	3.0

Ten cubic centimeters of each sample were irradiated for ninety minutes in quartz-covered, water-cooled dishes, and when tested for pressor action in a dog, showed the following:

1. Synephrin base, P_H 9.0 or above, develops a reddish brown color after irradiation; its action is not increased but is somewhat decreased.
2. The solutions of P_H 7.0 and lower are markedly increased in activity by ultraviolet irradiation. The color developed was a paler brown color than the P_H 9.0.
3. Solution of P_H 8.0 had an increased action but not as much as those of lower P_H.
4. Solutions of the whole P_H range had the same activity before irradiation.

This experiment shows that in order to develop the increased activity, synephrin must have an acid present. The amount of acid necessary to produce maximal effect is approximately the amount needed to form just completely the acid salt of the base present. It is left then to determine what rôle the acid plays in bringing about the formation of the active ingredient produced when synephrin is irradiated.

5. All samples become more acid when irradiated. Heating the irradiated solution with alcohol and concentrate H_2SO_4 gives the odor of ethyl acetate indicating the presence of acetic acid. The Table XXII shows the P_H before and after ninety minutes' irradiation and pressor activity of the irradiated drug. The change in P_H may be due to the acetic acid which is formed as an oxidation product of the irradiation.

TABLE XXII

P_H		ACTIVITY OF IRRADIATED DRUG ON DOG BLOOD PRESSURE (MM. HG.)		
BEFORE IRRADIATION	AFTER IRRADIATION	NORMAL	AFTER INJECTION	DIFF.
9.0	6.5	128	140	12
8.0	3.6	127	194	67
7.0	3.0	114	234	120
6.0	2.9	108	176	68
5.0	2.9	118	166	48
4.0	2.8	116	156	40
3.0	2.5	118	156	38

6. Solution of synephrin base when irradiated for seven hours had a slightly increased action on blood pressure and showed a P_H of 4.7.

DISCUSSION

Epinephrine

The literature on the effect of ultraviolet rays on epinephrine solutions is not very extensive. If we consider that ordinary daylight provides some ultraviolet rays originating from the sun, we may have a possible explanation of the changes taking place in dilute solutions of epinephrine salts commonly observed. Such dilute solutions are stated (N.N.R. 1933—p. 194) to rapidly lose their strength, the deterioration being accompanied by a reddish or brownish discoloration. For this reason, solutions of epinephrine are usually discarded as soon as any color develops although they may still be practically as active as when freshly prepared. There is considerable hesitancy about sterilizing an epinephrine solution by boiling for this same reason. It is possible that our opinion of the rapidity of destruction of epinephrine solution is somewhat exaggerated. Richter and Gerhartz[13] in 1908, while studying the action of roentgen rays on ferments, made a brief study of the action of soft and hard roentgen rays on solutions of epinephrine. By testing on the blood pressure of dogs, they found some evidence of destruction of pressor action, but there was too much variation to draw definite conclusions. Ludin[8] in 1918 stated that roentgen rays did not alter adrenalin action as judged by the action on the surviving intestine of rabbits. McGuigan and Mostrom[10] in 1913 reported the loss of glycosuric action of epinephrine boiled with sodium hydroxide, while some pressor action still remained. This work supported that of Ransom[12] in 1911. Savopol in 1914 exposed solutions of 1:1,000 adrenalin to the rays from a quartz mercury arc lamp and reported changes in some minor actions of adrenalin. 1. The hemoagglutinating and hemolytic properties were not diminished even with three hours' irradiation but rather increased to a certain extent. 2. The power of adrenalin to neu-

tralize tetanus toxin was lost by three hours of irradiation. 3. The necrotizing property of adrenalin on subcutaneous injection was attenuated with ten minutes' irradiation and entirely lost with one and one-half hours of irradiation. The effect of ultraviolet irradiation on the pressor action of epinephrine was reported by Verda, Kneer, and Burge in 1931. This method of judging the strength of epinephrine solutions is recognized by the U.S.P. X. After thirty-five minutes of irradiation, the pressor action was practically destroyed, and instead there appeared considerable depressor action. Ito and Terata also in 1931 reported an increase in the action of epinephrine solutions after irradiation for a short time and a loss of action by longer periods of irradiation. Another independent investigation by Ewing, Blickensdorfer, and McGuigan in 1931 on epinephrine and related products showed a loss of pressor action of epinephrine solutions, which was almost complete in samples irradiated ninety minutes. Kneer, Orth, Verda, and Burge in 1931 investigated the effect of ultraviolet irradiation and reported the loss of depressor action which nonirradiated solutions give when very dilute.

SYNEPHRIN

Since synephrin is a rather new drug, reports on its action are not very numerous. Tainter and Seidenfeld in 1930[19] made a quite thorough study of sympathomimetic compounds, including synephrin. They reported the stability of solutions of the drug to boiling, and studied its sympathomimetic action on blood pressure, blood vessels, intestine, bronchioles, and the property of chemosis. In 1931, Ewing, Blickensdorfer, and McGuigan first reported a marked increase in pressor action of synephrin salt solutions after irradiation for ninety minutes. Higgins, Ewing, and McGuigan in 1932[4] reported the effect of irradiated synephrin solutions on the dog heart—which effect could not be obtained with the nonirradiated drug. Stockton, Pace and Tainter in 1931[18] reported the clinical value of racemic synephrin in raising blood pressure, relieving asthmatic attacks, as an adjuvant to procaine in local anesthesia, and for shrinkage of mucous membranes with topical application.

OTHER DRUGS

Investigations of the action of ultraviolet light on drugs was stimulated by a rather extensive study of the formation of vitamin D. Rosenheim and Webster in 1928[14] showed that ergosterol alone, when treated photochemically (by ultraviolet light) produced vitamin D, and concluded therefore that ergosterol was the parent substance of this vitamin.

Pacini and McGuigan in 1930 reported the detoxification of 0.1 per cent solutions of nicotine when exposed to ultraviolet light. In 1931, Higgins, Ewing, and McGuigan showed that nicotine solutions were weakened by simple evaporation, but that when evaporation was prevented, ultraviolet rays still caused a destruction of nicotine. Wakeham and Tracy[21] in 1932 reported the effect of ultraviolet irradiation on 100 per cent and 10 per cent nicotine solutions. They reported a maximum destruction of toxicity in solutions irradiated one and one-fourth hours. Irradiation for a longer time resulted in an increase in toxicity. Gant[2] has recently published a paper on

the irradiation of nicotine showing that decomposition only takes place and that the decomposition products are finally nitrates, nitrites, and ammonia. Macht in 1932 reported the action of ultraviolet light on solutions of digitalis. He found a decrease in toxicity up to one hour and thirty minutes of irradiation, then an increase in toxicity up to two hours and fifteen minutes of irradiation followed by subsequent decrease.

Many of the conflicting or varying results of different investigators can be explained by the different methods used, either in irradiating or in methods of judging the activity of the drug. The first factor is the source and intensity of light used. This is usually stated in such investigations so that any one can readily evaluate this factor. The distance of the solution from the source of light is of equal importance in judging how much energy is actually reaching the substance irradiated. The volume of solution and surface area exposed to the rays has a great influence on the effect of the irradiation since the rays penetrate only slightly beneath the surface of the solution. Another factor of great importance is the concentration of the solution irradiated. The destruction of nicotine solutions reported by Pacini and McGuigan in 1930 was based on the irradiation of 10 c.c. of a 0.1 per cent solution in a Petri dish having an area of approximately 75 sq. cm. exposed to the rays. This means that 10 mgm. of nicotine were destroyed after ninety minutes of irradiation at 12 inches from the source. Wakeham and Tracy in their work on nicotine reported in 1932 irradiated 100 per cent and 10 per cent solutions in quartz tubes, the volume and surface area exposed not being stated. Their method of judging destruction of nicotine was by determining the M.L.D. in white rats. We point out these investigations as examples of differences in methods used and the inadequacy of some of these methods, which factor is probably the reason for the different results obtained. We have found that a 0.1 per cent solution of synephrin hydrochloride solution is markedly changed by ultraviolet irradiation whereas a 1.0 per cent solution irradiated under the same conditions is only slightly changed. Likewise epinephrine hydrochloride solutions in a 1:10,000 dilution are quite rapidly destroyed by the ultraviolet rays; whereas in a 1:1,000 dilution, very little change occurs under similar conditions. We have noted that the temperature of the irradiated solution also affects the results. At a higher temperature changes take place more rapidly presumably because these changes are usually photochemical reactions which are hastened by increased temperature.

In our work, we have confirmed previous investigations showing that ultraviolet light destroys the activity of epinephrine solutions as judged by various methods, and in no instance have we found any evidence of potentiation of this drug. Heating the solution of epinephrine hydrochloride with H_2O_2 produced similar changes, both chemically and in biologic activity. The chemical products resulting from oxidation explain the loss in activity.

The increased biologic activity of synephrin solutions effected by ultraviolet irradiation has been shown by various methods used in this investigation. Similar changes effected by heating with H_2O_2 lead us to believe that the

changes are only the result of oxidation. The exact nature of the hyperactive substance formed when solutions of synephrin salts are irradiated is difficult to ascertain, since a combination of unchanged synephrin with several oxidation products is present in the hyperactive solution. We hope in the near future to succeed in identifying this substance. The necessity of the presence of some acid with the synephrin base in developing the hyperactive substance is established.

The brief study of the hydrochloride of parahydroxyphenylpropanolamine gives evidence of changes similar to those with synephrin hydrochloride and indicates that the important change is probably in the phenyl group rather than in the side chain.

CONCLUSIONS

1. Epinephrine solutions are oxidized by ultraviolet light so that all normal actions of the drug are completely lost.

2. No evidence has been found to indicate any activation of epinephrine solutions by ultraviolet irradiation.

3. Some decomposition products of irradiated epinephrine have been identified.

4. Irradiation of solutions of synephrin salts increases their action many times as judged by all methods of standardization used.

5. Solutions of synephrin base do not exhibit any appreciable increase in action following irradiation.

6. Excess irradiation of hyperactive solutions of synephrin salts destroys their activity.

7. Irradiation of parahydroxyphenylpropanolamine increases its action approximately to the same extent as is found with synephrin salts.

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THE RÔLE OF AMIDOPYRINE IN THE ETIOLOGY OF GRANULOCYTOPENIA WITH SPECIAL REFERENCE TO ITS CHEMICAL STRUCTURE

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GRANULOCYTOPENIA (otherwise known as agranulocytosis, agranulocytic angina, or granulopenia) was first accurately described by Schultz[1] in 1922. Beckman[2] mentions the fact that the end-results of the disease were demonstrated by Pepper as long ago as 1857, but the blood picture was not recognized until Schultz's researches.

Beckman[2] gives an excellent description of the disease. He states that it is first characterized by a complete elimination of the myelocytes, followed in four days by immense reduction in the granular leucocytes, and later their complete absence. Two days later there are extreme symptoms of sudden collapse, chill, fever, injected pharynx or ulcerative stomatitis, sometimes jaundice, soon stupor or death (unless the maturation of myelocytes is resumed). General sepsis develops if the patient survives this stage, and death soon follows.

Benzene and the benzene ring are sometimes blamed for the causation of the disease. Kracke produced the disease in rabbits by injecting benzene into the circulation.[3] As benzene is rarely used as such in medicine, and then chiefly in the treatment of leucemia, and as the compounds bearing the benzene ring which we use therapeutically are well tolerated when given in proper doses (see the author's article on acetanilid[11]), I will show later why amidopyrine must be considered in a different category.

According to Madison and Squier,[4] 500 cases of granulocytopenia are now on record, the increase in the number of cases on record closely paralleling the increase in the use of amidopyrine within recent years. The authors ascribe the condition in 14 of their cases to "benzene ring derivatives," and later admit that they referred to amidopyrine. I am prepared to show that amidopyrine should not be classed as a benzene ring derivative.

Madison and Squier[5] in a later article give a detailed description of 14 cases of granulocytopenia, all of which followed the use of amidopyrine (7 with barbiturates, 6 alone, and 1 combined with other drugs). Eight patients died. The surprising part was that physiologic doses were used in all cases (one patient took amidopyrine gr. x daily for eleven days and developed the disease). The authors conclude their article as follows:

"(1) The increase in incidence of primary granulocytopenia (agranulocytic angina) has paralleled the increase in the use of drugs containing amidopyrine, and especially those containing amidopyrine with a barbiturate.

"(2) The disease has appeared most frequently in persons apt to be taking drugs: *physicians, nurses, and those directly under the care of a physician.*

"(3) In each of 14 patients the onset of primary granulocytopenia was directly preceded by the use of amidopyrine alone or in combination with a barbiturate.

"(4) The mortality in a group of 6 patients who continued the use of drugs containing amidopyrine was 100 per cent. In a group of 8 patients who did not continue the use of these drugs, only two died, and both of these died in the initial attack.

"(5) The administration of a single dose of amidopyrine to each of two patients who had recovered from the acute disease was followed by a rapid profound fall in the granulocytes.

"(6) One rabbit was given allylisopropylbarbituric acid with amidopyrine (allonal) by mouth in relatively large doses, showed an abrupt drop in granulocytes and died on the thirteenth day. Preceding death, there was complete absence of granulocytes in the peripheral blood; 17 other rabbits given allonal or amidopyrine showed no significant changes in the blood picture.

"(7) We believe that amidopyrine alone or in combination with a barbiturate is capable of producing primary granulocytopenia in certain individuals who have developed sensitivity to the drug.

"(8) We believe that the appearance of primary granulocytopenia following the use of such drugs may be the result of an allergic or anaphylactoid drug reaction."

Armand J. Quick, in a recent article,[6] and also in a letter[7] to the *Journal of the American Medical Association* states that drugs which produce allergy may perhaps cause a severe inflammatory reaction which may terminate in neurosis.

Hare[8] has collected 121 cases of untoward effects from antipyrine, most of which had erythematous patches and bullae. The dose was usually a moderate one. Amidopyrine is derived from antipyrine and is structurally similar.

Randall[9] describes a case of granulocytopenia following the use of barbiturates and amidopyrine. He blames the "benzene ring" in both drugs. I cannot agree with his conclusions as I will state in more detail later.

Zinniger[10] reports two cases of granulocytopenia of unknown origin and in a letter to the editor sent later, ascribes them to amytal compound (containing amidopyrine).

Hoffman, Butt, and Hickey[12] presented a series of 14 cases of granulocytopenia with 13 deaths. The one patient who recovered had developed the disease following dinitrophenol. The 13 who died developed the disease following the use of amidopyrine.

In November, 1933, Watkins[13] presented 32 cases of granulocytopenia which were observed at the Mayo Clinic. Of these 24 had taken amidopyrine or a barbiturate. Ten took amidopyrine alone and of these 2 died. One of the 8 who recovered had 12 attacks within two and a half years. He took amidopyrine for the relief of migraine and developed granulocytopenia after each session. One attack followed the use of ethyl methyl butyl barbiturate. Two patients used allonal, neither died; 5 used amytal, all died; 4 took sodium ethyl methyl butyl barbiturate, all died in the first attack. Two took phenobarbital, one died. Eight patients showed no definite history of drug taking, but had been under the care of physicians for chronic ailments, and it is thought that they had been taking either amidopyrine or the barbiturates. Watkins states: "Whether these persons have primary deficiency of the bone marrow is open to question, but before the onset of the neutropenic state, the leucocyte response in most instances had been normal. The fact that in two cases recurring neutropenia has regularly followed the use of amidopyrine, would indicate that in these cases there is apparent association between ingestion of the drug and neutropenia. The possible relationship between these drugs and granulocytopenia is not, in my opinion, a general contraindication to their use, but when they are to be used regularly, it would seem advisable to make a leucocyte count at intervals of every two or three days while the patient is in a hospital and every two or three weeks if the use of the drug is continued after the patient's dismissal from the hospital."

Hoffman, Butt, and Hickey[12] state also that they are conducting a series of experiments with rabbits. They state "amidopyrine alone was used in contrast to the combination of Madison and Squier of amidopyrine with allylisopropyl barbituric acid, because *amidopyrine was the only drug used in 13 of the 14 cases observed.*" They succeeded in producing a marked leucopenia in the rabbits ranging from 8 per cent to 20 per cent of normal.

The authors again blame the benzene ring for the leucopenia, but state "whether the latter (the benzene ring) is the actual toxic agent in the production of neutropenia needs further experimentation, part of which we are now undertaking. Our work points to amidopyrine as having a definite effect on myeloblastic tissue similar in man and rabbits. Whether this is an individual susceptibility of the nature of an allergic reaction as suggested by Pepper,[14] remains to be determined. *Until it is, the use certainly of amidopyrine alone or in combination with other drugs should be restricted to patients having leucocyte counts several times a week.*"

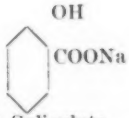
Beckman[2] is one of the few writers who seem to have correctly classified amidopyrine according to its structural formula, not as a benzene ring derivative, but *as a pyrazolon derivative.* It is true that there is a phenyl radicle present, but as this is a side chain of the pyrazolon nucleus, the pyrazolon is the active element in the drug.

Let us see the chemical nature of some of the antipyretics in common use. First we will consider derivatives of the benzene ring, or C_6H_6.

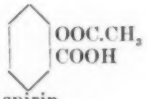
Benzene

$NH.COCH_3$
Acetanilid

OC_2H_5
$NHCOCH_3$
Phenacetin



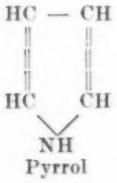
OH
$COONa$
Sodium Salicylate



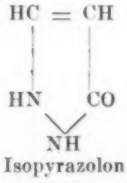
$OOC.CH_3$
$COOH$
Aspirin

All these compounds except benzene (which is not given therapeutically except in rare instances) are relatively nontoxic when given in therapeutic doses. Even in immense overdoses no cases of granulocytopenia have been traced to them. It is thought that acetanilid and phenacetin are gradually converted to para-mino-phenol which exerts an antipyretic action, and then excreted as the glycuronate or sulphate of the above compound.[15] Salicylates are excreted either as such or as modified glycuronates.

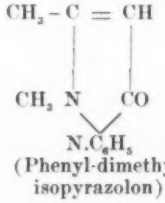
Amidopyrine and antipyrine belong to a different group. Instead of the hexagonal benzene ring, they are built about the pyrazolon ring, a modification of pyrrol, both being pentagons:



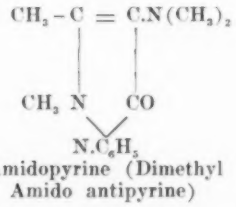
Pyrrol



Isopyrazolon



(Phenyl-dimethyl-isopyrazolon)



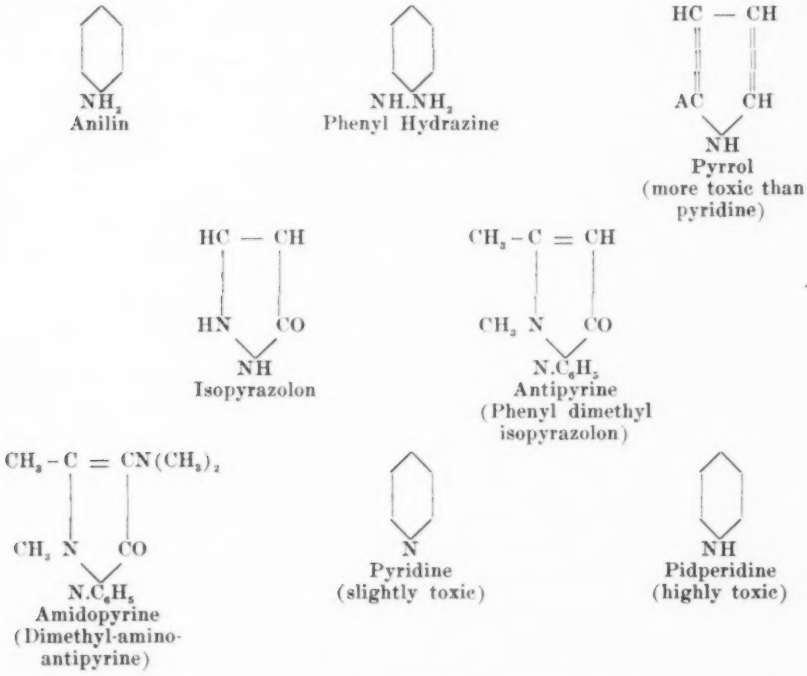
Amidopyrine (Dimethyl Amido antipyrine)

Why is amidopyrine more toxic than acetanilid, phenacetin or aspirin? I shall endeavor to answer that question from an analysis of its chemical structure. The fact that the pentagonal pyrrol ring instead of the hexagonal benzene ring is present is not the essential point, in my estimation. I consider the NH component of the ring as the toxic element, regardless of whether it remains as NH or whether the H is replaced by another radicle such as phenyl as in antipyrine or amidopyrine.

McGuigan[15] states "although antipyrine is derived from phenyl hydrazine, *which is a strong blood poison,* it is physiologically not a phenyl hydrazine

derivative, perhaps on account of the formation of the pyrazolon ring." McGuigan started with the correct idea, but as the toxicity of amidopyrine had not been fully appreciated, his final conclusion is not correct. McGuigan hit the nail on the head when he mentioned that antipyrine is derived from phenyl hydrazine. I maintain that it not only is derived from phenyl hydrazine, but partakes of its nature.

I will show several drugs having this NH or N radicle as an integral part of a ring:



Pyridine is slightly toxic. Piperidine is highly toxic and differs from pyridine solely in the substitution of an NH (imido) radicle for the N radicle. This shows the toxic character of the imide radicle. Furthermore, all alkaloids, such as morphine, nicotine, quinine, strychnine, cocaine, etc., contain this toxic radicle.

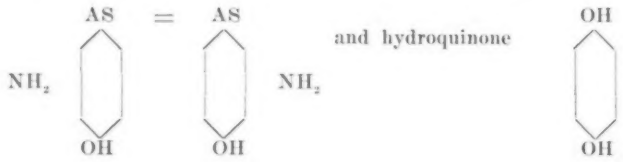
Amidopyrine and antipyrine have this imido radicle with the H replaced by a benzene ring, which apparently is insufficient to neutralize the toxicity of the imido radicle.

Phenyl hydrazine contains the imido radicle, not as an integral part of the ring, but as a closely joined side chain.

Examining other chemical combinations, we find the NH_2 (amino) radicle as a component of proteins, amino acids, and such end-products as urea and uric acid. It is nontoxic in most of its combinations, but a few compounds such as anilin, when not combined with side chains are toxic. Side chains decrease the toxicity to a marked extent, and permit of the use of accepted benzene derivatives with complete safety in medicine.

Barbituric acid and the barbiturates are products synthesized from urea and malonic acid, and present an incomplete purine ring—*not a benzene ring.* Uric acid may be synthesized in the laboratory from barbituric acid.

The writer does not maintain that amidopyrine is the sole cause, or that the imido radicle is solely responsible for the drug causation of granulocytopenia. He merely states that it is one of the important causes. Other drugs may likewise cause granulocytopenia, and the blame must be placed upon other equally toxic radicles. For instance dinitrophenol consists of a benzene ring with one (OH) and two (NO_2) radicles. The NO_2 radicles are quite as toxic as the NH. Picric acid is trinitrophenol, and is highly toxic. Other drugs which have caused granulocytopenia are salvarsan:



and likewise some of the barbiturates are said to have caused it.

Having demonstrated the toxic nature of amidopyrine, and having shown that it has produced more cases of granulocytopenia than any other drug in common use in medicine, the thinking members of the medical profession should consider amidopyrine and its progenitor antipyrine as too dangerous for use in medicine. Why should we use a drug with fear and trepidation, and be compelled to check its use with a leucocyte count every two days, as Watkins suggests, when we have safe and efficient drugs to answer our therapeutic requirements? There is no reason, in the light of present scientific investigation, ever to use the dangerous pyrazolon compounds, amidopyrine and antipyrine.

The writer[11] has shown that acetanilid is a safe and efficient drug when properly used. He has shown the unscientific nature of most of the adverse propaganda. Even the idea that caffeine should not be combined with acetanilid, as it was thought to form a toxic combination, has been exploded by McGuigan who showed by animal experimentation that caffeine decreases the toxicity of acetanilid. Other investigators have proved that citrates still further diminish its toxicity. A combination of acetanilid, caffeine, and potassium citrate would form an excellent and relatively nontoxic combination.

Lowy, and Helms, in a paper which I have had the privilege of reading, and which has not yet been published, based upon a questionnaire sent by Dr. Lowy to every hospital in the United States asking about the number of deaths, poisoning, and addiction from opiates, barbiturates, and amidopyrine, antipyrine, acetanilid, and phenacetin during a ten-year period shows the presence of far more cases of deaths and addictions from the barbiturates than from the antipyretics. There were practically no deaths from the latter group, but as granulocytopenia was then not generally recognized, and complete blood counts were not done in all cases, many deaths from unrecognized causes may

have been from granulocytopenia. Acetanilid, in spite of all the adverse propaganda against it, was rarely considered the cause of death, practically never as an addiction, and rarely as a cause of toxic phenomena.

Acetphenetidin is similar in its action to acetanilid but less efficient, and consequently requires large doses. In doses sufficient to produce results, it is more toxic than acetanilid.

CONCLUSIONS

1. Granulocytopenia has increased markedly in prevalence within the past few years. It is a virulent and rapidly fatal disease, and it has been proved beyond any reasonable doubt that amidopyrine is the most frequent cause of this condition.

2. Madison and Squier[5] have produced granulocytopenia in one dog by a combination of amidopyrine and allylisopropyl barbituric acid (allonal), while Hoffman, Butt, and Hickey[12] have produced granulocytopenia in several rabbits by feeding amidopyrine alone.

3. Amidopyrine is a pyrazolon compound containing the highly toxic NH (imido) radicle, is derived from phenyl hydrazine, and resembles the latter in its rapid reduction of granular leucocytes in the blood stream and bone marrow.

4. Fully 500 cases of granulocytopenia have been described within recent years, and the increase in its prevalence closely parallels the increase in the use of amidopyrine, the majority of patients having used it, the drug being practically always taken upon a physician's order.

5. The dangerous character of amidopyrine being now established beyond any reasonable doubt, amidopyrine should be strictly banned by the medical profession.

6. When anodynes or antipyretics are indicated, we have a safe drug[11] in acetanilid, which should be given in therapeutic doses, best combined with caffeine and potassium citrate. (An alternative but less efficient drug would be acetphenetidin.)*

7. By using the safer antipyretics mentioned, there is no doubt but that we will obtain greater efficiency, and rapidly eliminate granulocytopenia and other toxic phenomena now known to be caused by the dangerous pyrazolon group of drugs (amidopyrine and antipyrine).

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145 WEST SEVENTY-FIRST STREET

THE BACTERICIDAL AND FUNGICIDAL ACTION OF HOMOLOGOUS HALOGEN PHENOL DERIVATIVES AND ITS "QUASI-SPECIFIC" CHARACTER*

II. DERIVATIVES OF ORTHOCHLOROPHENOL (WITH NOTES ON CHLORINE-FREE ALKYL PHENOL DERIVATIVES)

EMIL KLARMANN, SC.D., VLADIMIR A. SHTERNOV, PH.D., AND LOUIS W. GATES, B.S., BLOOMFIELD, N. J.

INTRODUCTION

IN OUR preceding paper[1] we described certain regularities which we found in the relationship between the chemical constitution and the microbicidal action of aliphatic and aromatic substitution derivatives of parachlorophenol.† The present paper deals with the derivatives of orthochlorophenol in the manner of our previous work. In addition, some consideration is given herein to the halogen-free para-alkyl (and alkaryl) phenol derivatives, for two reasons; first to show what effect the introduction of chlorine into the ortho-position of the nucleus of para-alkyl phenols has upon the antibacterial action, and second to demonstrate the existence in this class of phenol derivatives of the "quasi-specific" effect previously described.

The derivatives of orthochlorophenol under consideration may be represented by the general formula:

where R is an aliphatic or aromatic substituent.

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*From the Plaut Research Laboratory, Lehn & Fink, Inc.
Received for publication, December 2, 1933.

†In this connection attention is called to another paper, dealing with bromophenol derivatives, which has been published in the meantime.[2]

The microorganisms used in this investigation, the methods of their cultivation, and the testing procedures were the same as described in our preceding paper.

PARA-ALKYL DERIVATIVES OF ORTHOCHLOROPHENOL

A perusal of Tables I to IV indicates a very similar relation between the molecular weight and the microbicidal action in the class of the para-alkyl

TABLE I

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL UPON ORGANISMS OF THE TYPHOID COLON GROUP

MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	Eberthella typhi		*Eberthella paradysenteriae*		*Salmonella schottmülleri (B. para typhosus B)*		*Escherichia coli*	
	I	II	I	II	I	II	I	II
o-Chlorophenol	1: 350	2.5	1: 350	2.3	1: 300	2.1	1: 300	2 3
p-Alkyl Derivatives								
Methyl	1: 1,000	6.3	1: 800	5.3	1: 700	5.4	1: 700	5.4
Ethyl	1: 2,750	17.2	1: 2,000	13.3	1: 3,500	25.0	1: 3,500	29.2
n-Propyl	1: 6,000	40.0	1: 6,000	40.0	1: 5,000	35.7	1: 4,000	33.3
n-Butyl	1:13,000	86.7	1:12,000	80.0	1:10,000	66.7	1: 5,000	38.4
n-Amyl	1:12,000	80.0	1:12,000	80.0	1: 6,000	40.0	1: 2,500	19.2
tert. Amyl	1: 4,500	32.1	1: 7,000	46.7	1: 3,000	21.4	(1: 2,500)	(17.9)
n-Hexyl	(1: 3,500)	(23.3)	----	--	----	--	----	--
n-Heptyl	(1: 2,500)	(16.7)	----	--	----	--	----	--
Aromatic Derivatives								
4-Benzyl	1: 5,000	35.7	1: 7,000	53.9	1:10,000	71.4	1: 3,500	26.9
6-Benzyl	1: 3,750	25.0	1: 5,000	38.5	1:10,000	71.4	1: 3,000	23.1
Phenol (Control)	1:140-150	1.0	1:140-160	1.0	1:140-150	1.0	1:140-150	1.0

TABLE II

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL UPON PATHOGENIC COCCI

MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	Staphylococcus aureus		*Streptococcus (hemol. strain)*		*Micrococcus catarrhalis*	
	I	II	I	II	I	II
o-Chlorophenol	1: 200	2.9	1: 200	2.0	1: 300	2.0
p-Alkyl Derivatives						
Methyl	1: 600	7.5	1: 500	5.6	1: 700	4.7
Ethyl	1: 1,100	15.7	1: 1,500	15.0	1: 2,500	16.7
n-Propyl	1: 2.250	32.1	1: 3,000	33.3	1: 4,000	26 7
n-Butyl	1: 7,500	93.8	1: 8,000	88.9	1:10,000	66.7
n-Amyl	1:20,000	286.0	1:20,000	222.0	1:20,000	133.0
tert. Amyl	1:10,000	125.0	1:11,000	122.0	1: 7,000	46.7
n-Hexyl	1:40,000	500.0	1:50,000	555.0	1:50,000	333.0
n-Heptyl	1:30,000	375.0	1:30,000	350.0	----	--
Aromatic Derivatives						
4-Benzyl	1:10,000	125.0	1:13,000	163.0	1:20,000	143.0
6-Benzyl	1: 5,500	68.8	1: 8,500	94.4	1:20,000	133.0
Phenol (Control)	1:70-80	1.0	1:80-90	1.0	1:140-160	1.0

derivatives of orthochlorophenol, as was found before in the class of ortho-alkyl derivatives of parachlorophenol.

The microbicidal potency with regard to the typhoid-colon group (*Eberthella typhi, Eberthella paradysenteriae, Salmonella schottmülleri, Escherichia coli*)

reaches its maximum with the n-butyl derivative and declines thereafter (Table I). However, with regard to all the other test organisms (Tables II, III, and IV) comprising cocci, acid-fast bacteria and pathogenic fungi, the n-amyl

TABLE III

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL UPON ACID-FAST BACTERIA

MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	Mycobacterium tuberculosis (hominis)		*Mycobacterium leprae (murium)*		*Mycobacterium smegmatis*	
	I	II	I	II	I	II
o-Chlorophenol	1: 200	2.2	1: 200	2.2	1: 200	2.2
p-Alkyl Derivatives						
Methyl	1: 500	5.6	1: 400	4.4	1: 500	6.3
Ethyl	1: 1,600	17.8	1: 1,600	17.8	1: 1,400	15.6
n-Propyl	1: 3,000	33.3	1: 3,000	30.0	1: 3,000	33.3
n-Butyl	1: 7,000	77.8	1: 7,000	77.8	1:10,000	125.0
n-Amyl	1:20,000	222.0	1:16,000	178.0	1:20,000	250.0
tert. Amyl	1:10,000	111.0	1: 8,000	88.9	1:11,000	138.0
n-Hexyl	1:16,000	178.0	1:20,000	222.0	1:40,000	500.0
n-Heptyl	1: 7,000	77.8	1:10,000	111.0	1:16,000	200.0
Aromatic Derivatives						
4-Benzyl	1:13,000	163.0	1:14,000	140.0	1:12,000	150.0
6-Benzyl	1:10,000	125.0	1:12,000	120.0	1: 8,000	100.0
Phenol (Control)	1:90-100	1.0	1:90-100	1.0	1:90-100	1.0

or the n-hexyl derivatives are the most potent; moreover their potency is of a higher order of magnitude. The latter compound which shows a maximum potency with regard to the three pathogenic cocci and a very considerable one (though not always a maximum) against the other microorganisms studied, is comparatively very little effective against *Eberthella typhi* and also against the other three bacteria of the typhoid-colon group; but no definite quantitative data could be obtained in reference to the latter microorganisms owing to wide fluctuations in the individual experiments.

TABLE IV

THE FUNGICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	Monilia albicans		*Trichophyton rosaceum*	
	I	II	I	II
o-Chlorophenol	1: 250	2.2	1: 200	2.2
p-Alkyl Derivatives				
Methyl	1: 750	8.3	1: 700	7.0
Ethyl	1: 2,000	22.2	1: 1,600	17.8
n-Propyl	1: 4,000	44.4	1: 3,750	41.7
n-Butyl	1: 8,000	88.9	1: 8,000	80.0
n-Amyl	1:25,000	278.0	1:20,000	200.0
tert. Amyl	1:10,000	100.0	1:10,000	90.9
n-Hexyl	1:25,000	278.0	1:16,000	160.0
n-Heptyl	1: 7,000	70.0	----	--
Aromatic Derivatives				
4-Benzyl	1:14,000	156.0	1:14,000	156.0
6-Benzyl	1: 8,000	88.9	1: 7,000	77.8
Phenol (Control)	1:90-110	1.0	1:90-110	1.0

Thus the "quasi-specific" effect again is in evidence, beginning with p-n-hexyl-o-chlorophenol which shows such considerable germicidal potency with regard to some microorganisms and so little of it with regard to others. The difference between the molecular weights of the compounds of maximum potency against the two groups of microorganisms into which this effect divides them, is narrow involving the step from the n-butyl to the n-amyl or n-hexyl derivative.

While there are in the series of orthochlorophenol derivatives several compounds of noteworthy germicidal and fungicidal potency, a comparison of the microbicidal action of the individual members of this series with that of the parachlorophenol series studied previously, reveals without exception a considerable superiority of the ortho-alkyl derivatives of parachlorophenol over

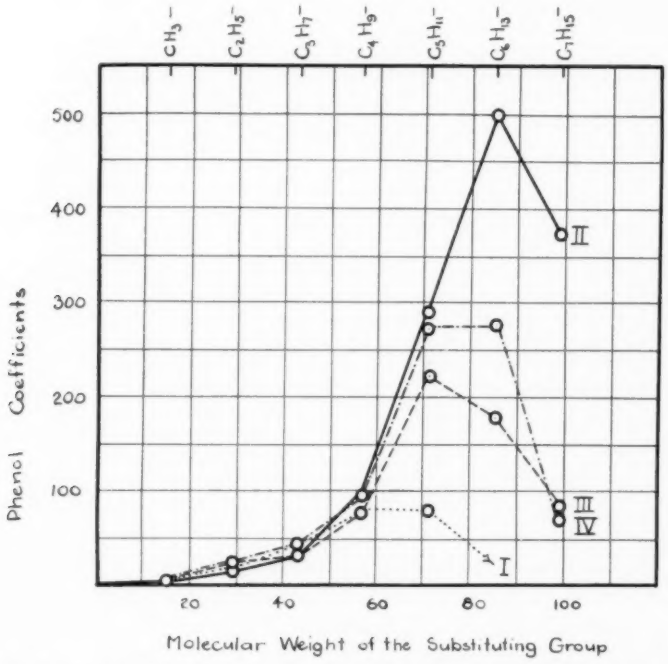

Fig. 1.—The germicidal action of para-alkyl derivatives of orthochlorophenol. Test organisms: I . . . . . *Eberthella typhi.* II———— *Staphylococcus aureus.* III - - - - - - *Mycobacterium tuberculosis (hom.).* IV ..... *Monilia albicans.*

the para-alkyl derivatives of orthochlorophenol. The same remarks apply to the p-benzyl-o-chlorophenol as compared with the o-benzyl-p-chlorophenol previously prepared. The o-benzyl-o-chlorophenol is less effective than the corresponding p-benzyl derivative.

For reasons of economy the discussion of Tables I to IV is limited to the above few remarks; the reader is referred to the tables themselves for further details. A graphic description of the conditions encountered in this series of compounds, illustrating the "quasi-specific" effect of the higher homologs is furnished by Fig. 1 in which the data obtained with one representative test-organism of each of the four groups studied have been used for the sake of greater clarity.

PARA-ALKYL PHENOL DERIVATIVES (CHLORINE-FREE)

While the antibacterial properties of the homologous series of phenol derivatives have been referred to by previous investigators,[3] it was deemed desirable to carry out a bacteriologic investigation of the para-alkyl phenol series with the aid of representative test-organisms and using the same experimental conditions in order to ascertain the effect of substitution by chlorine in the ortho-position to the hydroxy group upon the germicidal action.* Table V presents the results obtained in terms of minimum concentrations destructive of microbic life in ten minutes at 37° C., and the phenol coefficients calculated therefrom.

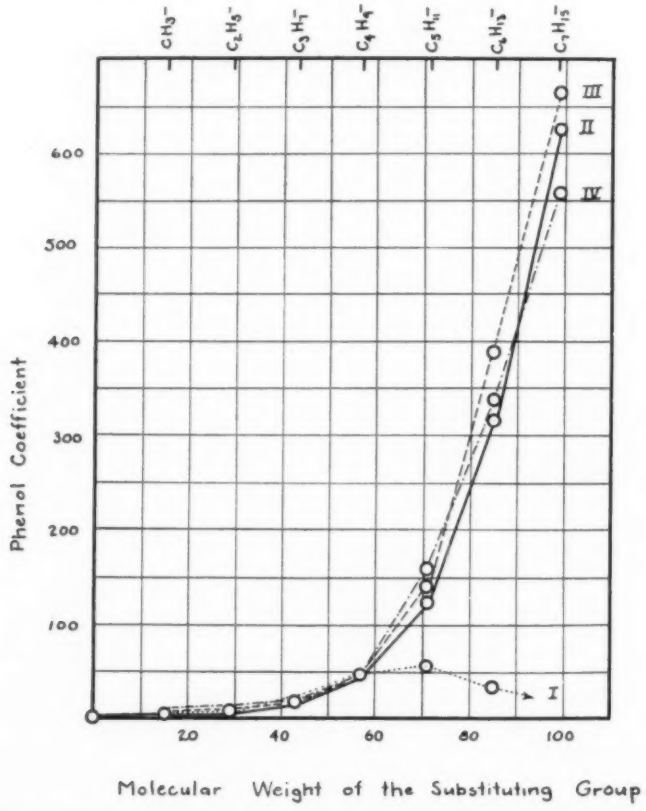

Fig. 2.—The germicidal action of para-alkyl derivatives of phenol (chlorine-free). Test organisms: I . . . . . *Eberthella typhi.* II———— *Staphylococcus aureus.* III - - - - - *Mycobacterium tuberculosis (hom.).* IV ..... *Monilia albicans.*

When compared with the respective columns in Tables I to IV it shows that with the exception of the homologs with the highest molecular weights the o-chloro derivatives are on the average about twice as effective as the chlorine-free para-alkyl phenols.

The phenomenon of "quasi-specific" action observed in the several series of halogen alkyl phenol derivatives is encountered also in the series of the

*These compounds were obtained by the reduction of the corresponding ketones by means of amalgamated zinc and hydrochloric acid; the ketones, in turn, were formed by an intramolecular rearrangement of the corresponding esters of phenol in the presence of aluminum chloride under definite conditions.

TABLE V

THE MICROBICIDAL ACTION OF PARA-ALKYL PHENOL DERIVATIVES (CHLORINE FREE)
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	Eberthella typhi		*Staphylococcus aureus*		*Mycobacterium tuberculosis (hominis)*		*Monilia albicans*	
	I	II	I	II	I	II	I	II
Phenol	1: 150	1.0	1: 80	1.0	1: 90	1.0	1: 90	1.0
Alkyl Derivatives								
Methyl	1: 300	2.0	1: 160	2.0	1: 200	2.2	1: 200	2.0
Ethyl	1:1,000	6.3	1: 500	6.3	1: 600	6.7	1: 700	7.8
n-Propyl	1:2,750	18.3	1: 1,300	16.3	1: 1,600	17.8	1: 1,600	17.8
n-Butyl	1:7,000	46.7	1: 3,500	43.7	1: 4,000	44.4	1: 4,000	44.4
n-Amyl	1:8,000	53.3	1:10,000	125.0	1:12,000	133.0	1:14,000	156.0
n-Hexyl	1:5,000	33.3	1:25,000	313.0	1:35,000	389.0	1:30,000	333.0
n-Heptyl	(1:2,500)	(16.7)	1:50,000	625.0	1:60,000	667.0	1:50,000	556.0

halogen-free para-alkyl phenols. Thus, with respect to *Eberthella typhi* the microbicidal potency increases at first with the increasing weight of the substituting radical, reaching a maximum in the case of the p-amyl phenol, and decreases thereafter, while with regard to *Staphylococcus aureus*, *Mycobacterium tuberculosis* (*hom.*), and *Monilia albicans* the increase is continuous without the evidence of a maximum being reached in the case of the n-heptyl derivative; the same compound shows comparatively very little efficacy against *Eberthella typhi*. These conditions are illustrated graphically in Fig. 2. The similarity in the course of the curves in Fig. 1 in this and the preceding paper† is remarkable and justifies the assumption that the "quasi-specific" effect is common to different homologous series of phenolic germicides. (By way of advance information, further support is lent to this idea by the findings made in the case of the three series of monoethers of dihydric phenols.)

TOXICITY

Using the same method as described in our preceding paper, we determined the toxicity of the p-n-alkyl compounds of the orthochlorophenol series and also of those of the series of chlorine-free p-alkyl phenols (Table VI). In the former

TABLE VI

TOXICOLOGIC DATA
SUBCUTANEOUS INJECTION IN MICE
MINIMUM LETHAL DOSES IN MILLIGRAMS PER GRAM OF BODY WEIGHT

	P-ALKYL DERIVATIVES OF O-CHLOROPHENOL	P-ALKYL DERIVATIVES OF PHENOL (CHLORINE FREE)
Phenol		0.45
o-Chlorophenol	0.7	
p-Alkyl Derivatives		
Methyl	1.5	0.50
Ethyl	4.0	1.0
n-Propyl	6.0	2.0
n-Butyl	15.0	3.0
n-Amyl	20.0	5.0
n-Hexyl	>20.0	6.0
n-Heptyl	>20.0	10.0

†And also in Fig. 1 of the paper dealing with bromophenol derivatives.[2]

series the minimum lethal doses are almost the same as found in the o-n-alkyl parachlorophenol series studied previously; more particularly, enormously large doses of the higher homologs appear to be tolerated by the animal organism. Thus the remarks made in our previous paper as to a potential utility of some of these compounds for chemotherapeutic purposes apply as well in the case of the series under consideration.

Table VI gives also the minimum fatal doses of the compounds of the (chlorine-free) p-n-alkyl phenol series. Here, too, the toxicity decreases sharply with the increasing weight of the substituting group; i.e., as the germicidal potency increases the toxicity decreases. A comparison of the chlorine substituted and the chlorine-free homologous series discloses that the introduction of the chlorine atom into the nucleus of a p-alkyl phenol is accompanied by a sharp drop of the toxicity (and, as indicated before, by a sharp increase in microbicidal potency).

SUMMARY

Continuing the studies on the bactericidal and fungicidal properties of halogen phenol derivatives, we investigated the homologous orthochlorophenol series with the aid of the same methods, and using the same test organisms as described in detail in our preceding paper.

As in the parachlorophenol series previously studied, so also in the orthochlorophenol series we found a number of compounds of a remarkable bactericidal and fungicidal potency. However, when compared with corresponding compounds of the parachlorophenol series, the aliphatic and aromatic derivatives of orthochlorophenol are less effective microbicides than the corresponding compounds of the former series.

The division of the microorganisms into two classes, on the basis of their susceptibility to the selective, "quasi-specific" action of the higher homologs, as observed in the case of the compounds of the parachlorophenol series, is effected by certain orthochlorophenol derivatives as well.

The bacteriologic study of the homologous series of the halogen-free p-alkyl compounds of phenol was included in this work in order to ascertain the effect of the introduction of chlorine upon the microbicidal action. The demonstrated existence of the "quasi-specific" effect in this series of phenol derivatives (and also in other series discussed elsewhere) permits the conclusion that this effect is common to different classes of phenolic germicides.

The toxicity to mice of the compounds of the homologous orthochlorophenol series agrees very closely with that of the corresponding parachlorophenol derivatives. Thus in the series under discussion, too, the increasing weight of the substituting radical not only produces an increase in microbicidal potency (within definite limits) but also a decrease of toxicity. A comparison with the chlorine-free p-alkyl phenol derivatives shows that the introduction of chlorine into the nucleus of p-alkyl phenols brings about a very decided drop in the toxicity.

We acknowledge gratefully the valuable assistance rendered by Mr. A. Grawehr, of our laboratory staff, in the bacteriologic part of this investigation.

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A STUDY OF COCCIDIOIDES IMMITIS*

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INTRODUCTION

THE patient from whom the organism *Coccidioides immitis* was isolated was admitted to the Veterans' Administration Hospital, Alexandria, La., Sept. 12, 1931, with diagnosis of pulmonary tuberculosis. The tubercle bacilli had not been found.

Report of finding the organism *Coccidioides immitis* was made Oct. 7, 1931. The patient was put on iodide treatments. Ten days later the routine sputum examination revealed *B. tuberculosis*, as did succeeding examinations. When first seen by the writer, the lesion of coccidioidal granuloma involved a small area with a diameter of about 1.0 cm., in the pharynx. Three months later the major part of the buccal cavity was filled with discharging sinuses. The patient was discharged from the hospital Jan. 15, 1932, and died at his home Mar. 7, 1932.

The patient had spent his entire life in Louisiana, except nine months' World War service, four months of which was overseas service. He had been a gas meter mender and had suffered nasal and pharyngeal irritation from gases while at his work. In December, 1930, he had made some metal lamps from junk material and recalled the extreme irritation due to the dust from the buffing wheel. The initial lesion appeared about six weeks later.

This statement is made because infection with this organism is rare, and in this country is confined almost exclusively to the San Joaquin Valley, California; also the factors in the onset of the disease have varied greatly. The only previous case report from the South was made by Lynch[1] in 1920.

GROWTH ON ARTIFICIAL MEDIA AND MICROSCOPIC FINDINGS

The organism *Coccidioides immitis* was first seen in this case in cultures and was found later in fresh and stained smears. The small lesion on the patient's pharynx had been scraped and several broth tubes inoculated. Within twenty-four hours a fluffy mass had formed which remained suspended in the clear medium for some time, but gradually it sank to the bottom of the tube. This mass was made up of a network of filaments and spores. (Fig. 3.)

Transfers were made from the culture tube to agar plates and carbohydrate broths. On the carbohydrate media the growth was rapid and abun-

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dant, but no acid or gas formed. At the end of forty-eight hours slightly raised white colonies had formed on the agar plates, and later an occasional brown colony was noticed. In the colonies were found small sporelike bodies which were gram-positive, nonacid and alcohol-fast. (Fig. 5-A.) Growing from the colonies were filaments which were branched and septate and contained

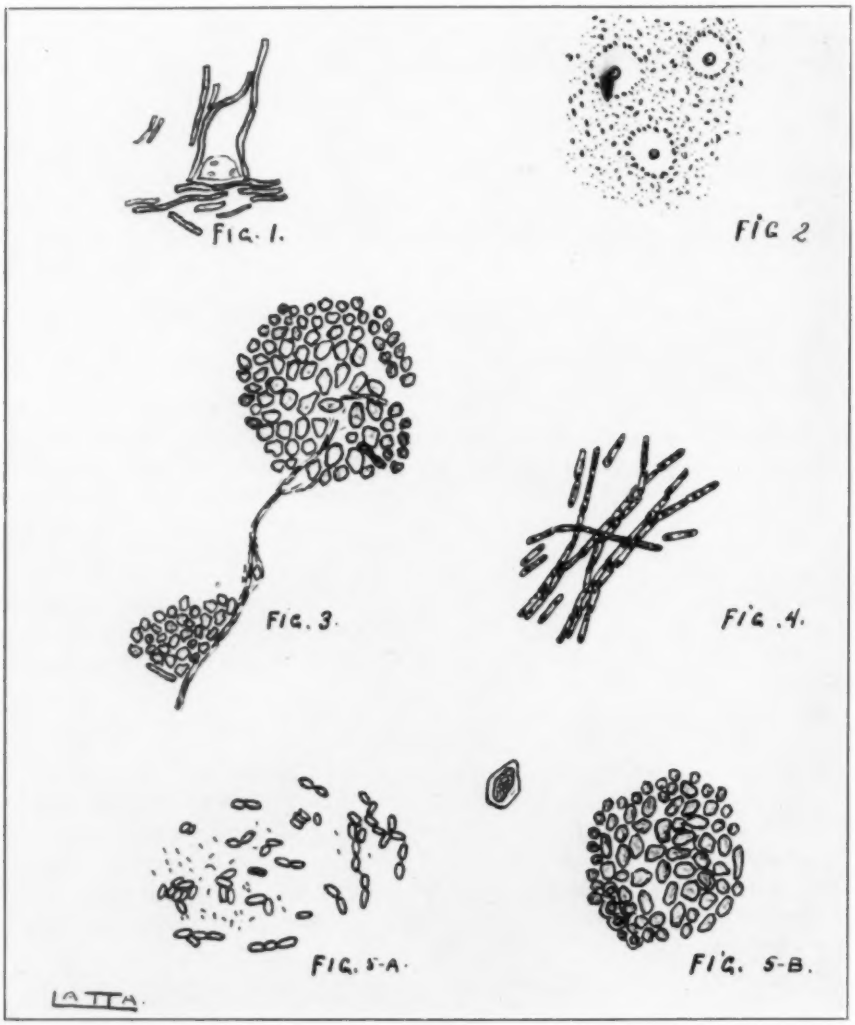


Fig. 1.—An empty spherical capsule and mesh of mycelium. (From animal tissue and culture forms.)

Fig. 2.—Double-contoured spherical forms. (From animal tissue.)

Fig. 3.—Broth. Hyphae and conidia.

Fig. 4.—Agar. Mycelium.

Fig. 5-*A*.—Agar. Spore forms.

Fig. 5-*B*.—An elliptical double-contoured form and spore forms. (From animal tissue and culture forms.)

bright, refractile vacuoles. (Fig. 4.) A loop of the mycelium was transferred to broth tubes and plain agar slants. In broth, characteristics were identical with former findings on this medium; on the agar slants aerial

hyphae developed after a few days and grew rapidly into a cloudy white covering. Culturally and microscopically the organism showed all the characteristics first described in detail by Wolbach.[2]

Cultures from the human lesion were kept growing for six months on ordinary laboratory media. Carbohydrate broths and Sabouraud's agar gave the most rapid and abundant growths. In liquid media flakes formed in the clear medium, or a grayish white, lacelike covering appeared on the surface; both forms slowly sank to the cottom of the tube. As the cultures grew old, this sediment changed in color from tan to dark brown. Broth tubes, some containing the original material, others subcultures, were allowed to evaporate in the incubator. These were transferred to the ice box for six weeks. Dextrose broth was added to the moist sediment and the tubes replaced in the incubator. The liquid changed from a dark brown to burgundy color within twenty-four hours, and a heavy network covered the surface.

On solid media the growth usually appeared as seen in agar cultures; small, discrete, white colonies, from which aerial hyphae, or deep penetrating mycelium, or both, grew. The colonies which remained discrete grew to several times the original diameter, often appeared in concentric circles with wrinkled surface and varied in color from cream to dark brown. When growing thickly, the small white colonies spread rapidly over the surface of the medium forming a creamy covering from which pure spore forms could be recovered. Aerial hyphae were most abundant, though delayed in development when grown at room temperature. Conidia, usually arranged at the end of hyphae, were observed in old cultures. Chlamydospores within the hyphae, described by Ophüls,[3] were found occasionally. They bore a marked resembalance to the spherical bodies recovered from the sputum and found in animal tissue.

Rixford and Gilchrist[4] first reported in this country the morphology of *Coccidioides immitis* as it appears in the tissues, a double-contoured spherical structure, varying from 5 to 60 microns or more in diameter. The double life cycle, spherical forms in the animal body, mycelia in artificial media, and their transformation, have been described by several writers. With a modification of the method of MacNeal and Taylor,[5] we studied the transformation of spherical bodies into mycelia. Fresh pus was taken from a lesion of the disease and suspended in broth. A loop of the suspension was placed on a flamed cover glass. A drop of agar was placed in a sterile hollow-ground slide. The cover glass was inverted over the well and sealed on the slide with paraffin. Several preparations were made, incubated, and examined daily. In some organisms no changes occurred, from others protoplasm flowed through the cell wall, branched and developed into a network of septate mycelium. As the culture grew old, the hyphae became clubbed, and spores formed, development previously observed in plate cultures.

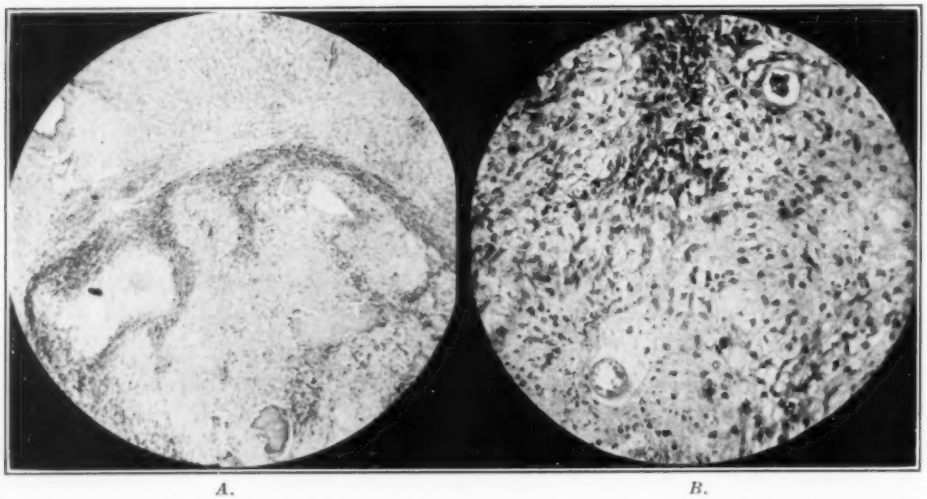
ANIMAL EXPERIMENTATION

It was known that *Coccidioides immitis* is pathogenic for rabbits, guinea pigs, and dogs. For the purpose of studying the organism in tissues and mak-

ing certain serologic examinations it was decided to reproduce the disease coccidioidal granuloma in experimental animals.

Three guinea pigs were each given a 0.5 c.c. intravenous injection of a killed mycelial growth of *Coccidioides immitis*, suspended in 0.85 per cent sodium chloride solution. The growth had been removed from an old agar culture in which aerial hyphae had grown so densely that the appearance was that of a light cotton covering. The guinea pigs were killed three weeks later. Pathologic findings were small firm masses on the abdominal wall, pinpoint lesions on the liver, a lumbar vertebra lesion, miliary nodules on the lungs and spleen, and red and swollen testicles. In smears and cultures *Coccidioides immitis* was found.

Histologic sections were made from the spleen and testes of one of the guinea pigs. The coccidioidal bodies stained readily with methylene blue, hematoxylin-eosin, iron-hematoxylin, and with Gram stain. These double-contoured



A. *B.*

Fig. 6.—*A*, Liver of rabbit through calcified necrotic area and cellular infiltration showing two coccidioidal bodies in the center. Low power. *B*, The same two bodies and granulomatous type of cells. The foreign body at upper right is of unusual view. Inner sac dense, packed spores, one of which can be seen escaping into the open space between the walls. High dry power.

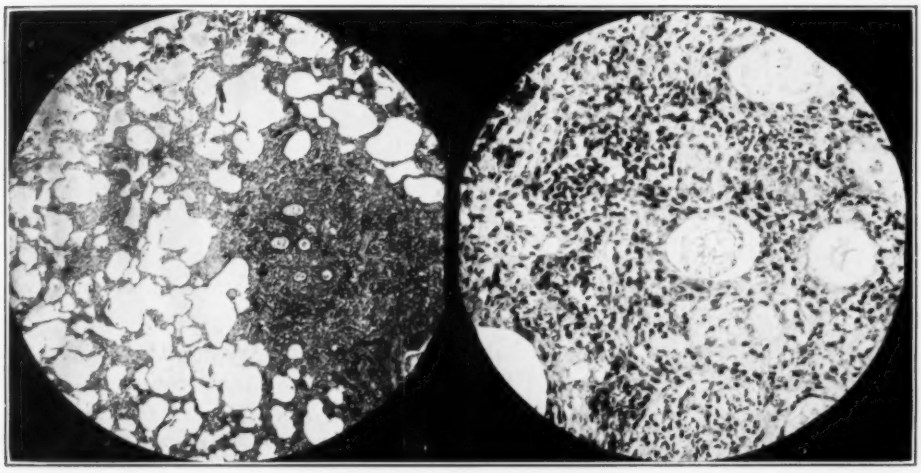
bodies varied greatly in size. No budding or mycelial growth occurred. Reproduction takes place by the formation of small intracellular spore bodies, which may be capsulated within the parent cell. The spore bodies are liberated by bursting capsule and grow out directly, either outside, or often still within old capsule, into young spherical forms.

An old agar culture of *Coccidioides immitis* from the patient's lesion was used for intraperitoneal inoculation of two rabbits. The heavy white mold was removed from agar plates, washed, and centrifuged in 0.85 per cent sodium chloride solution. The injection was made of 2.0 c.c. of the suspension of organisms, many rather elliptical forms, some round forms, and a few broken filaments.

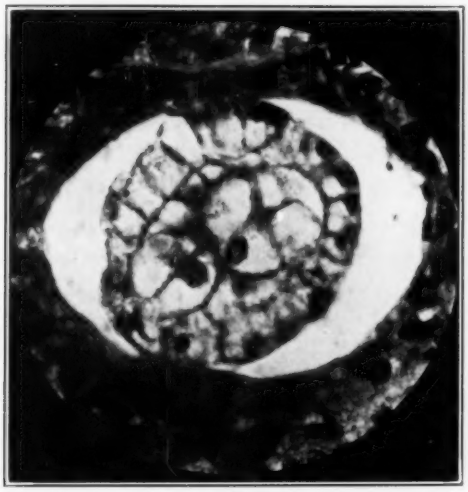
An antigen had been prepared for use in making complement fixation tests, precipitation and agglutination tests. The patient's lesion was aspi-

rated and a loop of the pus examined for spherical forms. The exudate was allowed to dry and was extracted with alcohol and ether. A grayish white powder was recovered and dissolved in 0.85 per cent sodium chloride solution.

Mixtures of the rabbit serum and the suspension were positive in precipitation tests in dilutions of from 1:20 to 1:240 and in dilutions from 1:20 to



A. *B.*



C.

Fig. 7.—*A*, Cat lung. There are large granulomatous areas showing several coccidioidal bodies. Low power. *B*, The same bodies photographed in *A*. In the upper right foreign body the inner wall has ruptured and sporulating forms are free in the outer sac. Just below is a small form in which the endospores are eccentrically placed in the inner wall. Farther below there is a large body which shows vacuoles and protoplasmic content in a double-contoured wall but no endospores. In the center of the field is a coccidioidal body, which is seen in detail in *C*. High dry power. *C*, Oil Immersion. The architecture of coccidioidal body near center of field Figs. *A* and *B*, spherical form, double-contoured a space between which does not stain, a vacuolated protoplasmic sac containing sporulating bodies.

1:320. The serums were negative for complement fixation tests and agglutination tests. With similar methods Davis,[6] who experimented with patient's serum, obtained positive complement fixation reactions. Davis and also Cooke[7]

had negative results with agglutination tests and positive results with precipitation tests.

After the second week, the rabbits rapidly lost weight and were killed at the end of the third week. One was examined. Nodules appeared on the peritoneal surface and about the intestines. Pearly nodules appeared on the liver; the spleen ruptured. Several of the pearly structures were removed from the liver, crushed between glass slides, moistened with 10 per cent sodium hydroxide and examined. The double-contoured bodies of *Coccidioides immitis* were found. In stained smear and cultures, the forms previously described were observed. Photographs and descriptions of the coccidioidal bodies in histologic sections from the rabbit's spleen are seen in Figs. 6A and 6B.

A very large house cat was given a 3 c.c. intraperitoneal injection from the same suspension from which the rabbit had been inoculated. Two weeks later nasal discharges were noticed. The serum was positive in precipitation tests in dilutions of from 1:20 to 1:80. The cat was killed at the end of three weeks. The lungs showed miliary lesions. The spleen ruptured. In smears from the lungs the highly refractile bodies and endosporulating forms of *Coccidioides immitis* were found. Histologic sections of the lungs were photographed. The photographs, also descriptions, of the coccidioidal bodies under different magnifications are seen in Figs. 7A, 7B, and 7C.

The experimental animals used in the work were fully grown, healthy males. Within two weeks after inoculation with the *Coccidioides immitis* organisms a change in physical condition could be noticed, and this change was pronounced by the end of the third week. Loss of weight, loss in strength, roughness and loss of hair were characteristic. The guinea pigs became highly nervous; the rabbits and cat were sluggish and indifferent.

SUMMARY

1. *Coccidioides immitis* is a fungus which has a double life cycle. It reproduces by mycelium in cultures and by endogenous spore formation in tissues.

2. *Coccidioides immitis* is the cause of a definite infectious disease, coccidioidal granuloma, in man.

3. The disease can be reproduced in the guinea pig, rabbit, and cat by intravenous and intraperitoneal injections of *Coccidioides immitis* organisms.

4. The serum of animals in which coccidioidal granuloma has been reproduced gave positive reactions in precipitation tests, negative reactions in the complement fixation and agglutination tests.

5. The organisms of *Coccidioides immitis* are found in pus, sputum, tissues of infected animals and can be recovered by culture and animal inoculation.

While the major part of this work was done after work hours, I wish to express appreciation for encouragement received from Dr. L. A. Walker, clinical director, who suggested that I write this report for publication; and Dr. J. B. Latta, pathologist, who made the excellent drawings of forms of *Coccidioides immitis* recovered from cultures.

Acknowledgment is made to the Veterans' Administration, Washington, D. C., for permission to publish this paper and for recognition of the work in the *Medical Bulletin*.[8]

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OBSERVATIONS ON THE GONOCOCCIDAL ACTION OF MALLOPHENE IN URINE*

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IT IS well known that the bactericidal action of antiseptics in urine does not parallel the action in water at the same dilution. As a rule a greater concentration is needed in urine than in water to obtain the same bactericidal action. Therefore, preference should be given to urines as a medium for dilution of the antiseptic for bactericidal tests when the antiseptic is proposed for clinical use in the treatment of infections of the urinary tract.

Gonococci are killed quickly in highly acid or highly alkaline urines. Therefore, when observations are not made near neutrality, careful controls should be made of the urine without the addition of antiseptics.

It may be seen from Table I that gonococci were killed in twenty minutes in dilution of 1-2,000 and 1-3,000 of mallophene in urine in all hydrogen ion concentrations from 4.9 to 7.0. In this observation the hydrogen ion readings were made before the urine was sterilized by boiling so that the actual hydrogen ion concentration in the test solutions were somewhat higher than recorded in Table I. A four-plus growth was obtained in the control of urine 6 whereas there was no growth in this urine containing the mallophene solutions.

Table II demonstrates that mallophene in a dilution of 1-4,000 did not kill the gonococci in twenty minutes in urines near the neutral point, but there was no growth after twenty minutes in the more acid urines containing mallophene. At least part of the bactericidal action in the urines with a high hydrogen ion concentration should be attributed to acidity so that it would seem that mallophene in a dilution of 1-3,000 is necessary to kill gonococci within twenty minutes near neutrality.

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Urines do not seem to have a distinct bactericidal action between pH 6.4 and 7.0. Below 5.4 all urines exhibit some bactericidal action to the gonococcus, but between 5.4 and 6.4 all urines are not equally bactericidal in proportion to acidity, and it is apparent that there are other factors in individual instances that influence bactericidal action in addition to acidity.

TABLE I

GONOCOCCIDAL TEST OF MALLOPHENE ADDED TO URINE*

DILUTION OF MALLOPHENE	URINE 1 pH 4.9	URINE 2 pH 5.4	URINE 3 pH 5.7	URINE 4 pH 5.8	URINE 5 pH 6.2	URINE 6 pH 7
1-2,000 0.5 c.c. 1-100 Mallophene and 9.5 c.c. Urine	0	0	0	0	0	0
1-3,000 0.5 c.c. 1-150 Mallophene and 9.5 c.c. Urine	0	0	0	0	0	0
Control (Urines Without Mallophene)	1+	2+	2+	2+	3+	4+

*Subcultures to solid mediums from various urines 20 minutes after addition of gonococcus suspension in urine 6.

TABLE II

MALLOPHENE 1-4,000 ADDED TO BOILED URINE AND GONOCOCCIDAL TESTS MADE

SPECIMEN	pH OF BOILED SPECIMEN	GROWTH AFTER SUBCULTURES		pH OF FRESH SPECIMEN
		5 MIN.	20 MIN.	
1	5.6	0	0	4.9
2	5.7	1+	0	5.1
3	6.1	0	0	5.7
4	6.6	1+	0	6.3
5	6.8	2+	1+	6.5
6	7.6	4+	3+	6.8
7	7.8	4+	3+	6.8
Control Urine Without Mallophene 5	6.8	4+	4+	6.5

TABLE III

GONOCOCCIDAL TEST WITH MALLOPHENE EXCRETED IN URINE OF PATIENT WITH NORMAL URINARY TRACT: SUBCULTURES 25 MINUTES AFTER GONOCOCCI WERE ADDED*

SPECIMEN	pH	GROWTH AFTER SUBCULTURE
1 Before Mallophene (Control)	5.4	0
1A 1 hr. After Two Mallophene Tablets	5.4	0
1B 2 hr. After Mallophene	5.6	0
1C 2 hr. 40 min. After Mallophene	6.0	2+
1D 3 hr. 10 min. After Mallophene	7.0	3+
2 Control	5.2	0
3 Control	5.6	3+
4 Control	6.7	4+

*One tablet of mallophene was given the patient at the time control specimen 1 was voided. One dram of sodium bicarbonate was given the patient after specimen 1B was voided.

An experiment was made to determine the antiseptic action of urine after the oral administration of mallophene and at various hydrogen ion concentrations. It may be noted in Table III that acidity alone killed the gonococci at a pH of 5.4 and below while there was growth in the presence of mallophene at a pH of 6.0 and above.

TABLE IV

BACTERICIDAL TESTS WITH EXCRETED MALLOPHENE URINES IN PATIENTS WITH GONORRHEA

PATIENT	pH	DURATION AFTER VOIDING UNTIL INOCULATION	RESULTS	DURATION AFTER VOIDING UNTIL INOCULATION	RESULTS	DURATION AFTER VOIDING UNTIL INOCULATION	RESULTS
1	5.1	10 min.	5 colonies	42 min.	0		
2	5.2	20 min.	50 colonies	30 min.	30 colonies	1 hr.	2 colonies
3	6.6	0	50 colonies	1 hr.	25 colonies		

Table IV illustrates the results by culture of the inoculation of shreds of the urine at various intervals. These patients had active gonococcus infections, and mallophene was administered by mouth. It is possible that gonococci in shreds are more protected from antiseptic action since in one instance there was moderate growth after thirty minutes with a hydrogen ion concentration of 5.2.

While the above experiment does not indicate a high germicidal action of excreted mallophene in urine, clinically, in some instances of posterior infection, there was clearing of the second urine prompt enough following the administration of mallophene to indicate that there is favorable action in posterior gonococcus urethritis, particularly during the course of disease when anteroposterior irrigations are not likely to be well tolerated. However, the excretion of antiseptics is less likely to be followed by complications during this stage of the disease than retrograde irrigations through the urethra. It is possible that benefit from the antiseptic excreted in the urine may be partially due to cellular stimulation since the dye type of antiseptics is said to have increased permeability.

THE EFFECT OF ALPHA DINITROPHENOL (1-2-4) ON BLOOD CHOLESTEROL IN MAN*

LUTHER F. GRANT, M.D., AND PURCELL G. SCHUBE, M.D., BOSTON, MASS.

ALPHA dinitrophenol (1-2-4) is a crystalline solid. It is slightly soluble in water, and more so in ether or alcohol. In ethylene or propylene glycol it will make a 2 per cent solution, and when dissolved in water to which one-half its weight of sodium bicarbonate has been added, it makes a 3 per cent solution. It has a yellow brown color at a P_H of about 8.1 and becomes colorless at a P_H of about 2.5. It has a melting point of 114° C.

SURVEY OF LITERATURE

In 1916 Barral and Martin[1] reported that when alpha dinitrophenol was inhaled in the form of a vapor or dust there frequently resulted headache, dizziness, vomiting, fever, and occasionally death. In 1917 Lutz and Baume[2, 3] studied the toxicology of the drug and its distribution in the organs and tissues. In 1918 Warthen[4] and Merklen and Malloizel[5] and in 1919 Quignard[6] reported additional studies upon this chemical substance. Interest in alpha dinitrophenol then lagged somewhat until 1931 when Magne, Mayer and Plantefol[7] published their studies which were closely followed by those of Cutting and Tainter[8] in 1932. Since then Magne, Mayer and their collaborators[9] and Cutting and Tainter[10] have published a number of interesting papers upon this substance.

In none of these studies has the effect of alpha dinitrophenol on blood cholesterol been reported. Inasmuch as this latter substance is of some importance in metabolism, it was felt that such a study was justified.

PHYSIOLOGIC PROPERTIES

The physiologic properties of alpha dinitrophenol can be enumerated as follows:

1. Alpha dinitrophenol, when given to pigeons, rabbits, rats, cats, dogs, and men, in doses ranging between 3 and 40 mg. per kilogram of body weight, by any route, produces a remarkable increase in body temperature, up to 6° or 7° C., in conjunction with marked respiratory stimulation.

2. The maximum febrile response occurs about one hour after injection or several hours after oral administration.

3. The increased temperature can occur independently of the skeletal muscles or central nervous system.

*From the Psychiatric Clinic, Boston State Hospital.
Received for publication, January 17, 1934.

4. The temperature is not influenced by full doses of ergotamine, adrenalectomy or thyroidectomy.

5. The respiratory stimulation may occur independently of the pyrexia.

6. The pulse rate is increased.

7. The blood pressure shows no consistent change.

8. The nitrogen excretion is less than the nitrogen intake.

9. The organic acid excretion is not increased.

10. The weight is decreased.

11. The metabolic rate is increased as much as from 25 to 50 per cent.

12. Recovery, if the dose is not fatal, requires four or more hours.

13. Death may occur as a result of direct circulatory depression, hyperpyrexia, acidosis or anoxemia, dependent on the dose, rate of injection, etc., of drug.

METHOD

Fourteen patients were selected at random from a relatively large group for the study. Of these one had to be dropped because of uncooperativeness. Blood cholesterol estimations were obtained on each of the patients once a week for a period of three weeks before the administration of the drug. In this manner a fairly satisfactory cross-section of the existing blood cholesterol and its variations in each of the patients was obtained. The greatest range was 30 mg. per 100 c.c. This greatest range of variation of cholesterol was used in each case of this group as the "normal" variation for that individual. It was felt that the greatest variation and not the average variation should be used in order to eliminate the existence in our results of individual false positive findings.

The patients were given alpha dinitrophenol, gr. 5, by mouth, daily, for a period of twenty days. The weight and blood cholesterol estimations of these individuals were obtained before, during, and after the administration of the chemical compound. The method of estimation of blood cholesterol was that devised by Schube.[11] The results obtained were compiled in Tables I and II.

RESULTS

In this group of cases used for study the only physical pathology clinically observed was valvular heart disease (Case 6) and syphilis (Case 11). From a study of the data obtained it was felt that this pathology did not alter the value of the results. The age range of the cases was fairly extensive (from twenty-two to fifty-nine years) but this, too, did not seem to affect the results in any significant manner. The weight of each of the individuals in this series was considered to be within normal limits for that subject at the beginning of the study. After the administration of the alpha dinitrophenol in three subjects (Cases 10, 11, 14), there were definite gains of 5, 12, and 7 pounds, respectively. The largest definite loss of weight was 4 pounds in each of the two subjects (Cases 5, 8). In the remainder of the cases, although the weight fluctuated in some instances, it remained essentially at its original level (Table

TABLE I

CASE	AGE	C = BL. CHOL., W = WEIGHT	DURATION IN DAYS														
			0	1	7	14	20	21	23	26	30	33	37	40	41	53
1	35	C		90	102	105	Alpha dinitrophenol started—gr. 5 daily	166	150	133		125		100	Alpha dinitrophenol stopped	115
		W	125						125		125		127			
2	29	C		166	140			150		86		105		166		
		W	127						127		130		126			
4	59	C		125	122	122		133	150	160		181				
		W	142						143		143		142			
5	26	C		112	95	86		209	157	181		166		68		116
		W	125						126		126		122			
6	22	C		80	110	95		142	200	250		125		90		90
		W	118						117				118			
7	56	C		166	158	163		136	100	80		180				
		W	140						139		141		139			
8	29	C		100	105	86		130	125	130		80		95		100
		W	137						138				134			
9	32	C			94	117		124	153	107		153		100		83
		W	139						138		144		137			
10	39	C		87	95	95		214	166	150		118		86		95
		W	158						158		162		163			
11	57	C		105	79	96		171	166	160		100		148		107
		W	172						172		183		184			
12	50	C		83	82	74			127	77		95				
		W	132						132		132		132			
13	34	C		125	136	130		140	230	160		124		111		133
		W	124						125		124		125			
14	33	C		140	130	145		187	130	83		118		100		100
		W	120						121				128			

I). These observations are opposed to other reports upon the effect of alpha dinitrophenol upon body weight.

The blood cholesterol in each of these cases, irrespective of alterations in the weight, showed a deviation from normal. This deviation was usually in a positive direction. The range of this deviation was not the same for all cases. In some the blood cholesterol, after a preliminary rise, dropped below "normal" before stabilizing itself within its original range (Table I).

The blood cholesterol values during the administration of alpha dinitrophenol are compared with the average normal blood cholesterol values in Table II. The largest positive change was 155 mg. and the smallest 13 mg. Of the group, four showed an increase in blood cholesterol of 100 mg. or more; seven had values of from 33 to 100 mg.; and two had values of 33 mg. or less. The largest rise occurred on the first day in five cases, on the third day in four cases, and on the thirteenth day in three cases. The largest negative change was –82 mg.

TABLE II

CASE NO.	AVERAGE NORMAL CHOLESTEROL	BLOOD CHOLESTEROL DURING ALPHA DINITROPHENOL—MG. PER 100 C.C.							
		HIGHEST VALUE			LOWEST VALUE			AVERAGE	DIFFERENCE OF AVERAGE
		AMT.	DIFF.	DA.	AMT.	DIFF.	DA.		
1	99	166	67	1	100	1	20	139	40
2	153	166	13	13	86	–67	6	129	–24
4	123	181	58	13	133	–	–	155	32
5	98	209	111	1	68	–30	20	156	58
6	95	250	155	6	70	–25	20	161	66
7	162	180	18	13	80	–82	6	124	–38
8	97	130	33	1	80	–17	13	112	15
9	106	153	47	3	100	– 6	20	127	21
10	92	214	122	1	86	– 6	20	147	55
11	93	166	73	3	100	7	13	149	56
12	80	127	47	3	77	– 3	6	100	20
13	130	230	100	3	111	–19	20	153	23
14	138	187	49	1	83	–55	6	124	–14

and the smallest was –3 mg. Of these negative changes, three were –50 mg. or more; two were from –25 to –50 mg.; two were from –15 to –25 mg.; and three were from 0 to –15 mg. The largest negative values occurred on the sixth day in four cases, on the thirteenth day in one case, and on the twentieth day in five cases. Thus in most of the cases, there is an initial elevation of blood cholesterol, then a drop to below the original level followed by a gradual return to "normal."

The largest average positive change was 66 mg. and the largest average negative change was –38 mg. There were ten cases with positive average values which ranged from 15 to 66 mg., four of which were 50 mg. or more, one between 40 and 50 mg., one between 30 and 40 mg., and four between 20 and 30 mg. There were three cases with negative average values, –14 mg., –24 mg., and –38 mg.

From a study of these figures and Tables I and II it can be stated that the blood cholesterol is altered during the administration of alpha dinitrophenol. We are unable to correlate or explain this alteration. Further studies are being conducted in an attempt to do these things.

SUMMARY

Alpha dinitrophenol was administered by mouth to 13 adult men of normal weight. The weight and blood cholesterol values of these individuals were studied and presented.

NOTE: No cutaneous lesions or toxic symptoms were observed.

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UROBILINURIA—FALSE EHRLICH REACTION CAUSED BY PYRIDIUM MEDICATION*

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RECENTLY it was demonstrated by one of us in a patient receiving pyridium (Phenylazo-2-6-diamino-pyridine monohydrochloride—Merck and Co.) by mouth that this drug causes a false positive urobilinuria reaction when tested with Ehrlich's reagent (paradimethylaminobenzaldehyde). The fact that the dilution units were much above the highest reported pathologic urobilin findings prompted an investigation of the probable cause of this result. When pyridium was dissolved in water and tested with the acid solution of Ehrlich's reagent it was found to yield the characteristic red color of urobilin.

Aqueous and urobilin-free urine solutions of pyridium were tested in varying dilutions with Ehrlich's reagent. It was possible to detect a color change that would have been called "positive" in dilutions of either solution up to 1 part in 75,000. Goerner and Haley[1] have shown that the concentration of pyridium in the urine of a patient receiving 0.6 gm. by mouth daily is upward of 1 part in 3,000. The addition of HCl in the same concentration as is used in Ehrlich's reagent (1 part concentrated to 2 parts water) caused the urine or water solution of the dye to assume the characteristic color change. The change in color on testing with Ehrlich's reagent, then, is due merely to the change in P_H of the solution of the aniline dye, pyridium. Alkalinization of the solutions causes the color to change to yellow, with the formation of a crystalline precipitate. On microscopic examination this precipitate is seen to be composed of needlelike crystals.

L. D. Scott[2] recently proposed a modification of the Ehrlich test, which he claims will not give false positive reactions due to drugs excreted by the kidneys. He recommends the use of butyl alcohol to extract the coloration caused by the reaction between urobilin or urobilinogen and Ehrlich's reagent. He states, "A measured amount of urine is treated with an appropriate amount of a specially prepared solution of paradimethylaminobenzaldehyde. After being allowed to stand for a given time, the mixture is heated under standard conditions in a boiling water-bath for a short period, and after cooling the coloration so produced is extracted with amyl or butyl alcohol. This extracted red coloration, which is practically free from foreign material and

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other interfering colors, is then compared in the colorimeter against a standard ferric thiocyanate solution almost identical with that employed by van den Bergh for the estimation of bilirubin in blood." Aqueous and urine solutions of pyridium were subjected to the test as he specified. The dye itself is extractable with butyl alcohol and consequently will give the same characteristic red color in the extract. That the color is due entirely to the acid was made apparent here also by the substitution of HCl for the Ehrlich's reagent.

When tested with Schlessinger's urobilin reagent, pyridium did not give a positive reaction.

The fact that the pyridium on excretion via the genitourinary tract is not chemically identical with the compound as ingested was ignored in our laboratory experiments. We first showed that the excreted product did give a positive reaction. Also, Goerner and Haley[1] demonstrated that by colorimetric comparison the ratio of the pure drug to the excretant is 30:33.5. The color reaction is the only thing of interest here, and the two substances are almost identical in that respect.

Urines which were shown to contain urobilin by both Ehrlich's and Schlessinger's tests were treated with HCl alone, and the color change did not occur. It is, therefore, quite easy to detect this drug reaction by the addition of HCl. We have made it routine that all urines which come to our laboratory and are shown to contain urobilin by Ehrlich's test be tested for color changes on the addition of HCl alone.

SUMMARY

The laboratory report of unusually high concentration of urobilin in a patient's urine led to the discovery that pyridium excreted in the urine gives a positive reaction with Ehrlich's reagent.

This positive reaction, a red or pink color, was found to be present in dilutions of the drug well above that in which it is commonly excreted in the urine.

The pink color was shown to be due entirely to the acid in the solution of Ehrlich's reagent.

Scott's modification of the test failed to remove this false reaction, in that the dye itself is extractable in butyl alcohol.

Schlessinger's test for urobilin does not give a positive reaction with pyridium.

This false reaction may be detected by treating all urines which are positive to Ehrlich's test with HCl alone. A pink or red color with acid indicates the presence of the dye. Discontinuing the pyridium for three or four days[3] is apparently the only means of obtaining a true test for urobilin with the Ehrlich reagent.

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LABORATORY METHODS

A COMPARATIVE STUDY OF THE KELLY TEST AND THE FRIEDMAN MODIFICATION OF THE ZONDEK-ASCHHEIM TEST FOR PREGNANCY*

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INTRODUCTION

THE influence of the anterior pituitary hormone on the immature ovary and the application of this influence in the diagnosis of pregnancy has been described by Zondek and Aschheim.[1] Since the publication of their paper a number of modifications of their technic as well as several other tests have been described in the literature. Among the latter, that of Kelly[2] is one of the more recent. This test depends upon the fact that urine from pregnant women will cause the premature opening of the vaginal orifice of immature rats, whereas, normally the vaginal orifice remains closed until sexual maturity is reached. In view of the fact that the test as described by Kelly suggested several possible advantages as compared with the Friedman[3] modification of the Zondek-Aschheim test it seemed desirable to make a comparative study of the two tests under similar conditions.

METHODS

The technic followed in the test in which rats were used was basically the same as that proposed by Kelly; but, instead of giving two 5 c.c. injections, one in the morning and one in the afternoon of the same day, it was found satisfactory to give one 2½ c.c. injection on each of two successive days. The final examination of the rats was made at the end of ninety-six hours. This modification was tested in a number of preliminary experiments and found satisfactory. The urine was made neutral to litmus, filtered, and warmed approximately to body temperature before the intraperitoneal injections were made. Catheterized specimens were used in most instances. The technic used in the Friedman test consisted of the intravenous injection into rabbits of from 10 to 15 c.c. of neutralized urine on each of two successive days. The rabbits were killed forty-eight hours after the first injection and the ovaries examined. Both tests were made

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on the same specimen, but were made independently of each other so that the results of one test could not influence the decision on the other test. The results of both tests were later compared with the final diagnoses as recorded in the hospital records.

RESULTS

The results obtained from the comparative study of the two pregnancy tests are shown in Table I. A total of 59 cases were studied. According to subsequent histories as shown in the hospital records, there were 19 cases of pregnancy and 40 cases in which there was no pregnancy. It will be seen in Table I that the Friedman test showed 20 positives, whereas the Kelly test showed 21 positives. The error in the Friedman test was found to be due to the retention of at least part of the placenta after an abortion, which is known to give a positive result. One of the two errors in the Kelly test was made on the same specimen which gave the error in the Friedman test and is subject to the same explanation. The other error occurred in a case in which the patient was suffering from an ovarian cyst. The Friedman test on this same specimen was negative. It is generally accepted that the Friedman test depends upon the presence in pregnancy urine of anterior pituitary hormone (anterior pituitary-like hormone, Collip, et al.[4]). The Kelly test, however, is apparently due directly to the increased secretion of the female sex hormone by the rat as a result of the stimulation of its ovaries by the anterior pituitary hormone found in pregnancy urine. Also, it is well known that the injection into an immature rat of female sex hormone alone will cause the premature opening of the vaginal orifice.

TABLE I

COMPARISON OF RESULTS OBTAINED FROM THE TWO LABORATORY TESTS WITH THE CLINICAL FINDINGS IN 59 CASES

	FRIEDMAN TEST	KELLY TEST	CLINICAL FINDINGS
Positive	20*	21*	19
Negative	38	29	40
Animals died; test not repeated	1	9	
Totals	59	59	59

*See text.

In certain pathologic conditions of nonpregnant women sufficient female sex hormone may be excreted in the urine to produce a premature opening of the vaginal orifice in the rat, and thus give a false positive in the Kelly test. Perhaps the second error in the Kelly test was the result of increased secretion by the cystic ovaries so that sufficiently large quantities of the female sex hormone were excreted to bring about the changes observed. The absence of abnormally large amounts of anterior pituitary hormone resulted in a negative Friedman test. However, there were several other cases of ovarian cyst in which negative results were obtained with the Kelly test. Perhaps in these latter cases the cysts were not of the type which cause an increased secretion of the female sex hormone. The 38 negative results from the Friedman test were correct, and one

experimental animal died in a test which was not repeated. The 29 negative results from the Kelly test were correct, and nine pairs of rats died in tests which were not repeated.

The earliest pregnancy to give a positive test was one in which the last menstrual period began approximately one month before the test was made. Another case gave positive results approximately six weeks after the onset of the last menstrual period. There were several cases of pregnancies of two months' duration, and various others ranging up to seven months' duration.

In connection with the Friedman test it was observed in three cases that rabbits which were probably too young gave false negative results; positive results were obtained upon repetition of the test with older animals. In each of these cases the rabbit was of a rather large strain developed for meat purposes, and was as large as rabbits considerably older but of a smaller strain. It is quite probable that these young rabbits would show positive results from pregnancy urine provided sufficient time was allowed—more than 48 hours. It seems evident that fully mature animals will give a positive reaction in much less time than is required for younger animals, and for that reason are preferable. In all cases in which animals were used which were known to be mature the results agreed with the clinical findings.

In one case which had been previously diagnosed as hydatidiform mole both the Friedman and the Kelly tests gave negative results, which were contrary to those expected. However, upon reexamination it was found that the mole had sloughed, which fact accounts for the results obtained.

The Kelly test has several advantages when compared with the Friedman test, as pointed out by Kelly.[2] They are: ease of handling the animals, economy in purchasing or raising rats as compared with rabbits, and the fact that it is unnecessary to kill or operate on the test animals to observe the results. However, the authors found the following disadvantages: approximately double the time is required, certain pathologic conditions in which an abnormally large amount of female sex hormone is excreted in the urine may give false positive results, and, unless adequate steps are taken to prevent it, the mortality rate among rats is likely to be high as a result of the intraperitoneal injections.

CONCLUSIONS

1. The Kelly test for pregnancy was found to compare favorably in accuracy with the Friedman modification of the Zondek-Aschheim test in a series of 59 cases, the results having been checked against the clinical findings in each case.

2. It appears probable that certain pathologic conditions affecting the ovaries directly may give a false positive reaction in the Kelly test, whereas the Friedman test will be negative under the same conditions.

3. Since the injections of urine in the Kelly test are made intraperitoneally, contaminated specimens are more apt to kill the experimental animals than is usually true when the injections are made intravenously, as in the Friedman test.

4. The Kelly test was found to offer no important advantage over the Friedman test except in the lower cost and the greater ease of handling the smaller animals.

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A MICROMETHOD FOR THE ESTIMATION OF HEMOGLOBIN*

Modification of the Benzidine Reaction

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THE blue color obtained when benzidine, acetic acid, and hydrogen peroxide are added to hemoglobin may be used as a measure of its concentration. On the basis of this reaction a microcolorimetric hemoglobin method has been devised. While this work was in progress, the method of Bing and Baker[1] appeared, which was a modification of a less desirable method of Wu.[2] These authors did not attempt to standardize the blue color but used the port wine color obtained on standing in the presence of rather strong acetic acid.

The present paper reports briefly the conditions under which the blue color may be used as a quantitative measure of hemoglobin. The method has certain advantages for with a very simple benzidine purification a preparation is obtained which gives straight line proportionality between color development and hemoglobin concentration. This proportionality was not obtained originally with the Bing and Baker method but Bing[3] has apparently corrected this by further purification. Also a permanent artificial standard is described which should aid in routine estimations where it is not convenient to do oxygen capacities for a standard hemoglobin solution. The time for color development is decreased to thirty-five minutes.

SOLUTIONS

Benzidine.—Purification: Add 5 gm. of a good grade benzidine base to 1 liter of water in a 2 liter beaker and cover with a watch glass. Dissolve by boiling for a short period. Add 2 or 3 gm. of carbon black (Norit) and boil for ten minutes. Filter while hot through two layers of filter paper, taking care that all the carbon is removed. Cool in ice water or allow to remain overnight in the ice box. Filter the solution through a Buchner funnel and dry in a desiccator kept in the dark. Preserve the dry benzidine in a brown bottle.

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Solution, 0.02 per cent: Weigh 0.100 gm. of the powdered benzidine on an accurate balance. Transfer to a 1 liter beaker, add 500 c.c. water and cover with a watch glass. Dissolve by boiling for a very few minutes. Cool and dilute to 500 c.c. in a volumetric flask. Filter and preserve in a brown bottle. It is stable for at least two months.

Acetic acid, 0.25 per cent: Prepare 1 per cent acetic acid by pipetting accurately 1 c.c. of glacial acetic acid into a 100 c.c. volumetric flask and diluting to mark. If there is any doubt about the strength of the glacial acetic acid, check the 1 per cent solution by titration (10 c.c. of acid requires 16.67 c.c. of N/10 NaOH, using phenolphthalein as indicator).

Prepare 0.25 per cent acetic acid as needed by accurately diluting 1 volume of the 1 per cent acetic acid with three volumes of water. (The 0.25 per cent acetic acid does not keep well because of mold growth.)

Hydrogen peroxide, 1 per cent: Accurately pipette 1 c.c. of 30 per cent hydrogen peroxide into a 50 c.c. graduated cylinder. Dilute accurately to 30 c.c. This may be preserved, in an electric ice box for a month, providing it is exposed only while being used. The 30 per cent hydrogen peroxide should be checked occasionally by titrating the 1 per cent solution with permanganate. (To a flask add 2 c.c. of 1 per cent hydrogen peroxide, about 10 c.c. of water, and 10 c.c. of 10 per cent sulphuric acid. Titrate to first pink color with N/10 $KMnO_4$. Per cent hydrogen peroxide $= T \times 0.085$, where T equals c.c. permanganate titration.) Correct if not 1 per cent.

It is important that the three solutions above be accurately made as a moderate variation in their strength appreciably affects the color development. The distilled water should be free of ammonium salts, sulphates, and chlorides, and if not should be redistilled.

Artificial Standard.—Ferric ammonium sulphate, 1 per cent: Weigh accurately 1 gm. of clean, clear crystals of the salt. Dissolve in water and dilute to 100 c.c. in a volumetric flask.

Potassium ferrocyanide, 1 per cent: Prepare accurately in same manner as the ferric ammonium sulphate.

Gum ghatti, 1 per cent: Add 1 gm. to 100 c.c. of warm water and allow to dissolve overnight. Filter through 2 or 3 layers of gauze.

To a 500 c.c. volumetric flask add exactly 6.5 c.c. of the 1 per cent potassium ferrocyanide solution. Dilute with water to about 450 c.c. Add 5 c.c. of the 1 per cent gum ghatti solution. Add exactly 10 c.c. of the 1 per cent ferric ammonium sulphate solution. Dilute to mark and mix. Allow to stand forty-eight hours before using. This solution is kept in the ice box. It is very stable and solutions over one year old have maintained their original color concentration. Under conditions of repeated experiments in our laboratory this solution is equivalent to the color produced by a concentration of 10 gm. of hemoglobin per 100 c.c. of blood when analyzed by the method proposed. One may check the strength of the artificial standard by doing oxygen capacities on blood. If necessary to modify the strength of the standard do so by adding more or less water while keeping the other constituents as directed.

METHOD

Prepare a 1 to 1,000 dilution of blood. This may be accomplished in any way suitable to conditions. Using the ordinary hemoglobin pipette (carefully calibrated), deliver 0.02 c.c. into exactly 20 c.c. of water. From an ordinary red blood cell counting pipette which dilutes 1-200,* 0.2 c.c. is added to 0.8 c.c. of water in a test tube. If only a small amount of blood is available, a special capillary pipette may be prepared to deliver 1 c.mm. of blood directly into 1 c.c. of water in the test tube. For larger amounts of blood the Folin micro sugar pipette, calibrated for 0.1 c.c. is convenient, diluting to 100 c.c.

Transfer 1 c.c. of the 1-1,000 dilution to a clean dry test tube. (A 50 per cent solution of acetic acid is convenient to wash previously used tubes.) Add 15 c.c. of the 0.02 per cent benzidine solution. Add 3 c.c. of the 0.25 per cent acetic acid. Add 1 c.c. of the 1 per cent hydrogen peroxide. Mix by inversion and place in a beaker of ice water containing plenty of ice. Allow to remain from thirty-five to forty minutes. Do not remove tube until ready to read. Place some of the solution in the left colorimeter cup and read the artificial standard in the right cup. It is important that the colorimeter cups and plungers are very clean. Wash with 10 per cent acetic acid, then distilled water and finally the solutions to be read.

Calculation:

X = gm. Hb. per 100 c.c. of blood.
S = reading of standard.
When unknown is set at 10 mm.
$X = S$.

EXPERIMENTAL

The usual experiments of varying the amounts and concentrations of the three principal reagents, benzidine, acetic acid, and hydrogen peroxide, were made. Too strong benzidine caused a precipitate to form while weaker solutions gave less intensity of color. If stronger acetic acid was used, the color intensity was less while with weaker acid the color was more unstable. One per cent hydrogen peroxide gave the maximum color. With both stronger and weaker solutions the color developed was less.

Bloods were prepared with concentrations of hemoglobin varying from 4 gm. to 20 gm. per 100 c.c. and the proportionality of color concentration was a straight line when read against the artificial standard.

It was found that the maximum color development was reached in about thirty-five minutes when it remained constant for about twenty minutes and then faded or became slightly purple.

The proper color concentration of the artificial standard was found by determining carefully the hemoglobin concentration of a sample of blood by its oxygen capacity $\times$ 0.746. By varying the strength of the standard a color concentration was produced which exactly matched that of 10 gm. Hb. per 100 c.c. of blood when analyzed according to method.

*The ordinary Hayems fluid cannot be used as diluent. An isotonic solution containing 0.65 per cent NaCl plus 0.15 per cent sodium oxalate is satisfactory.

The hemoglobin contents of twenty bloods were determined by their oxygen capacities and by the present method. The variation between the methods was not greater than 4 per cent.

Emphasis must be placed on the necessity of cleanliness and accuracy in preparing reagents. If conditions are carefully followed, it is believed that the use of the artificial standard will be very satisfactory for routine work.

SUMMARY

The blue color obtained when benzidine, acetic acid and hydrogen peroxide are added to hemoglobin has been standardized as a microhemoglobin method.

An artificial standard has been prepared for routine work.

The hemoglobin content of 1 c.mm. of blood may be determined.

The author wishes to extend his thanks to Dr. W. G. Karr for advice and suggestions during the progress of this work.

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THE BACTERICIDAL POWER OF BLOOD*

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IN a recent publication Frederick W. Shaw[1] discusses the question of the influence of the bactericidal power of the blood on the technic of taking blood cultures. He shows from citations of the literature that immune serum is not bactericidal but offers a satisfactory culture medium for bacteria. From his own experience in which he used defibrinated blood of the patient himself as a culture medium, he believes that this is a satisfactory procedure and that the natural bactericidal† property of the blood can safely be neglected.

I have no doubt that when there are sufficient microorganisms in the blood to survive the first hours of incubation when the bactericidal power of the blood is at its highest, positive blood cultures can often be obtained by this technic. However, experience with blood cultures, and in particular some experiments performed in 1931 indicate that when the numbers of organisms per cubic centimeter of blood is not large, the natural bactericidal power of the blood may very well interfere with obtaining positive results.

The experiments in question were not undertaken to answer directly the question discussed above. They were incidental control observations in the study of the bactericidal power of an antiseptic which it was thought might be used intravenously in bacteremias. As the study with the antiseptic was negative, the experiments were not published at the time. But the control observations necessitated the answering of certain definite questions, namely: 1. Is citration or defibrination preferable for experimental work? 2. Does citration impair the normal bactericidal power of the blood? 3. How bactericidal is normal blood for *Streptococcus viridans?* 4. For how long does the bactericidal effect of the blood continue? 5. How bactericidal is the patient's blood in subacute endocarditis for his own organisms and for organisms derived from other sources?

1. Is citration or defibrination preferable for experimental work (with bactericidal drugs)?

A *Streptococcus viridans* culture (No. 90) obtained from a case of bacterial endocarditis was used. It grew very rapidly, emulsifying well on shaking. Of a twenty-four-hour culture diluted 1:1,000, 1 c.c. was placed in each of two small flasks at the patient's bedside. One of the flasks contained a number of glass beads and the other 0.3 c.c. of 10 per cent sodium citrate. The patient had a case of polycythemia. Twenty cubic centimeters of blood were obtained from the patient and 10 c.c. put into each flask. The two flasks were then shaken

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†In the following report the word "bactericidal" is not used in the immunologic sense as for example in speaking of the Pfeiffer phenomenon, an amboceptor-complement reaction: it is used in the broad sense of a phenomenon which causes apparent disappearance of bacteria from the blood.

equally for ten minutes. Then 0.1 c.c. was removed from each flask, mixed in 5 c.c. of bouillon, and then plated with 10 c.c. of agar. After twenty-four hours the plate from the citrated blood showed 800 colonies, that from the defibrinated blood, 150 colonies. It was apparent that defibrination had removed a considerable number of bacteria, which were probably in the clot. For purposes of blood culture, particularly for immediate subcultures of blood on different media, and for the present experiments, citration would appear preferable to defibrination.

2. Does citration, as compared with defibrination, impair the normal bactericidal power of the blood? The technic was similar to that in the preceding experiment (blood was used from a patient with chronic cholecystitis) except that the bacteria were added to both flasks after instead of before defibrination. Dilution of the culture was higher, 1:20,000, in order to have a smaller number of colonies per cubic centimeter. Subcultures of 0.1 c.c. and 0.5 c.c. of blood from each flask were made at once and after the flasks had been in the thermostat one hour at 37° C. The results, read after forty-eight hours' incubation of the plates (in order to give all the colonies time to appear), are shown in Table I (the numbers indicate colonies per plate).

TABLE I

		SUBCULTURES AT ONCE	SUBCULTURES AT 1 HR.
Citrated blood	0.1 c.c.	32	21
	0.5 c.c.	137	35
Defibrinated blood	0.1 c.c.	42	12
	0.5 c.c.	126	51

The bactericidal effect is seen to be about as pronounced at one hour in the citrated as in the defibrinated blood. It is evident then that the addition of a small amount of sodium citrate does not impair the normal bactericidal power of blood.

3. How bactericidal is normal blood for *Streptococcus viridans*, and (4) how long does the bactericidal effect of blood continue?

This was determined on numerous occasions and showed very wide variation. In general it can be said that with different strains of *Streptococcus viridans* after one hour's incubation, about one-third to one-half of the organisms present disappear. After two hours' incubation about one-half to two-thirds or more disappear. After twenty-four hours in many instances 90 per cent or more of the microorganisms disappear from the blood. In other cases the bactericidal effect appears exhausted at this time and rapid multiplication of the bacteria ensues. In the following experiments 0.1 c.c. of a 1:15,000 dilution of a twenty-four-hour culture of a strain of *Streptococcus viridans* was added to 4 c.c. portions, respectively, of normal citrated blood and of bouillon. At once and again after two and one-half hours' incubation in the thermostat and after twenty-four hours at room temperature, subcultures of 0.1 c.c. each were plated and the colonies counted. The results are shown in Table II.

TABLE II

	COLONIES PER PLATE		
	SUBCULTURE AT ONCE	SUBCULTURE AT 2½ HR.	SUBCULTURE AT 24 HR.
Blood	512	52	6
Bouillon	480	692	Uncountable

Two similar experiments (both with normal blood, one with a hemolytic streptococcus) in which the primary bactericidal period was followed by rapid growth, are shown in Tables III and IV.

TABLE III

	HEMOLYTIC STREPTOCOCCUS		
	SUBCULTURE AT ONCE	SUBCULTURE AT 1 HR.	SUBCULTURE AT 24 HR.
Blood	115	14	Uncountable
Bouillon	80	100	Uncountable

TABLE IV

	STREPTOCOCCUS VIRIDANS		
	SUBCULTURE AT ONCE	SUBCULTURE AT 1 HR.	SUBCULTURE AT 24 HR.
Blood	125	65	Uncountable
Bouillon	65	100	Uncountable

5. How bactericidal is the patient's blood in subacute bacterial endocarditis for his own organisms and for organisms derived from other sources?

A rather high bactericidal power of the blood of cases of bacterial endocarditis for the homologous organism was observed in a number of experiments. When the number of organisms at the start was small, they were often killed off entirely in a few hours. Thus the following experiments were done with the blood of patients in the active stage of bacterial endocarditis.

To 5 c.c. portions of the patient's citrated blood were added 0.5 c.c., respectively, (a) of a heterologous *Streptococcus viridans* (strain No. 90), (b) of the patient's own *Streptococcus viridans* (strain No. 70), and (c) of sterile bouillon as control. At once, again after one hour, and after three hours, 0.5 c.c. of blood was plated and the colonies counted. By chance the number of organisms of strain No. 90 was too large at the start so that no count could be made, but it was obvious from the three-hour plate that this strain had multiplied vigorously. On the other hand, with the patient's own strain the large number of added organisms was greatly reduced at three hours, and in the sample of the patient's blood to which no further organisms were added the small number originally present (11 per 0.5 c.c.) were completely killed off.

TABLE V

	SUBCULTURE AT ONCE	SUBCULTURE AT 1 HR.	SUBCULTURE AT 3 HR.
(a) Blood plus Culture No. 90 (heterologous)	Uncountable	Uncountable	Uncountable but increased
(b) Blood plus Culture No. 70 (homologous)	50,000 (approximate)	3200 (approximate)	1000
(c) Blood without added bacteria	11	2	0

In the following experiment the bactericidal effects of a normal blood and of the blood of a patient suffering from bacterial endocarditis were compared on three strains of *Streptococcus viridans*, one of which (No. 14) was derived from the patient herself. To 4 c.c. portions of citrated blood was added 0.1 c.c. of a 1:20,000 dilution of a twenty-four-hour bouillon culture of each organism; 0.5 c.c. amounts of blood were plated.

TABLE VI

	CULTURE ADDED	SUBCULTURE AT ONCE	SUBCULTURE AT 2 HR.
Normal blood	No. 90	Uncountable	Uncountable but increased
	No. 70	2400	800
	No. 14	3400	1600
Patient's blood	No. 90 (heter)	2200	1200
	No. 70 (heter)	2400	500
	No. 14 (auto)	3200	220
	No addition of bacteria	250	85

It is seen that the patient's bactericidal power for the heterologous strain (No. 70) is of about the same order as that of normal blood. The initial growth of strain No. 90, another heterologous strain, in the patient's blood seems to have been inhibited as compared with its growth in normal blood; here it appears as though the inhibitory effect of the immune blood on a heterologous strain manifested itself in the poured plate. The patient's blood evidently reduced the number of her own added organisms (strain No. 14) at two hours very much more than did the normal blood. It appears that the inhibitory power of normal blood can be overwhelmed if the initial number of organisms is too great. Strain No. 90 had previously been shown to be quite susceptible to the normal bactericidal power of the blood when not initially present in excessive numbers; it was the strain used in most of the preceding experiments.

SUMMARY

1. Defibrination removes large numbers of bacteria from the fluid portion of the blood. They are probably held in the clot.

2. Sodium citrate does not impair the normal bactericidal power of the blood.

3. On incubation of the blood for a few hours, bacteria (*Streptococcus viridans* and hemolytic streptococcus) disappear in large numbers from normal blood, and in even larger numbers from the blood of patients infected with the homologous strain. The question whether this phenomenon is due to antibodies, phagocytosis, or disintegration of leucocytes with liberation of bactericidal substances, is not discussed.

4. The determination of bactericidal effects depends largely on the initial number of bacteria present. When this is very small, the bacteria may be completely killed off. When it is very large, the bactericidal effect may be completely lost.

CONCLUSIONS

The above experiments indicate that the normal and the immune bactericidal power of the whole blood cannot safely be disregarded and that the factor of

dilution probably plays an important rôle in the results obtained in blood cultures. They also show how carefully any experiments on chemotherapy of bacterial infections of blood need to be controlled.

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A METHOD FOR QUANTITATING THE REDUCING SUBSTANCE OF THE ANTERIOR HYPOPHYSIS*

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CHROMOPHILE adenomas of the hypophysis and aqueous extracts of the anterior lobes of animals contain a substance which actively reduces methylene blue, and which is not found in chromophobe adenomas or in extracts of other organs.[1] Hemoglobin does, of course, reduce methylene blue, but the reduction produced by anterior hypophysis substance occurs in the absence of hemoglobin. The present investigation was intended to study the chemistry of this reaction further and to determine which chemical fraction of the crude anterior lobe extract contained the reducing substance. A mildly alkaline extract made according to Evans' original formula,[2] which had a high reducing activity, was used as a starting point.

The method used for studying the activity of various preparations was based upon Ahlgren's technic.[3] The procedure consists in determining the time required to bleach a standard amount of methylene blue contained in an evacuated Thunberg tube under arbitrarily selected conditions.

Effect of Acidity.—It was found that between P_H 10 and P_H 8, little change in reducing time occurred; but at more acid reactions, the reducing effect fell off very rapidly. Below P_H 6, reduction was negligible. Accordingly, our test solutions were buffered at P_H 8 with Sorensen phosphate buffer, the final concentration of which was made up to about 0.06 molar.

Effect of Concentration of Methylene Blue.—At constant temperature and constant acidity, the time required by a known concentration of pituitary extract to effect complete bleaching was a direct linear function of the methylene blue concentration. This relationship failed, however, when such excessive amounts of dye were used that the reducing capacity of the pituitary substance was exceeded.

Electrolyte Concentration.—This had no appreciable effect within wide limits at the temperature and acidity employed.

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*From the laboratories of the Collis P. Huntington Memorial Hospital, Boston, and the Department of Neuropathology, Harvard Medical School.

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Effect of Temperature.—The effect of temperatures below 50° C. is uncertain because of the possibility of bacterial contamination which in some experiments gave an apparent optimum close to 40° C. Above 60° C., however, bleaching proceeded with increasing rapidity as higher temperatures were employed. Thus in one experiment the bleaching time at 80° C. was treble that at 60° C. In practice, it was found that reducing time could conveniently be read at 100° C. in solutions which failed to bleach completely at 40° C. even after forty-eight hours. Accordingly, bleaching activity was measured regularly at 100° C. by immersing the vacuum tubes in a bath of gently boiling water.

It should be emphasized that this method is a dynamic one, based upon rate of reaction. It was found that comparable results could be obtained by a titration method in which the amount of dye to be reduced was varied. This procedure, however, proved much more tedious and less accurate than the one employed.

Detailed Technic.—Into each Thunberg tube was placed 0.1 c.c. of 1:1000 methylene blue and 0.15 c.c. of molar phosphate buffer at P_H 8.0. Then the solution to be tested (adjusted to P_H 8.0) was added, and the total volume made up to 2.5 c.c. with distilled water. The tube was then evacuated as described by Ahlgren by means of a Cenco vacuum oil pump protected by a trap. Evacuation was begun cautiously until the initial frothing had subsided, then continued until (after about three minutes) brisk boiling had subsided and the tube had become perceptibly chilled (to the hand). It was then placed in boiling water and the time noted. Observations were made every half minute until complete bleaching was noted by comparing with a previously bleached control.

Standard Bleaching Curve.—A sterile alkaline extract of anterior pituitary substance,* made according to a modification of Evans' method, was arbitrarily selected as standard. The total volume was 2.5 c.c. The respective bleaching times for successive volumes of gland extract is shown in Table I.

TABLE I

VOLUME IN 2.5 C.C.	BLEACHING TIME IN MIN.
0.2 c.c.	45
0.3 c.c.	30
0.4 c.c.	19
0.5 c.c.	15
0.6 c.c.	11
0.7 c.c.	9
0.8 c.c.	6
1.0 c.c.	6
1.2 c.c.	4
1.4 c.c.	4
1.6 c.c.	3

Unit of Activity of Gland Extract.—An "alpha unit" was arbitrarily selected to serve as a measure of the apparent potency of various extracts. It was arbitrarily decided that an amount of extract containing 10α would

*Kindly provided by Parke, Davis & Company.

reduce the standard amount of methylene blue in fifteen minutes. Final dilutions in each case were adjusted so that the reducing time would approach fifteen minutes.

Extracts Tested.—To begin with, various commercial extracts of the posterior lobe and preparations from pregnancy urine containing an estrogenic substance supposedly of hypophyseal origin were tested and found to have no effect upon methylene blue.

Next, various globulin fractions of the crude alkaline extract were tested, as it is well known that the growth-stimulating hormone tends to remain with the globulin fraction. It was found that any chemical manipulation tends to decrease the reducing activity of a given extract. Since hemoglobin reduces methylene blue, the crude extract was brought to P_H 5.0, the hemoglobin filtered off, and the globulin precipitate redissolved at P_H 7.5. To give a concrete instance, one sample of crude extract contained 67*a* units and 6.3 mg. nitrogen per c.c. The filtrate (containing hemoglobin) showed 16*a* units and 0.5 mg. nitrogen per c.c. The precipitate, redissolved and made up to volume, contained 12*a* units and 4.2 mg. nitrogen per c.c. This solution was again precipitated in a similar manner and the precipitate redissolved and made up to volume when it showed 11*a* units and 3.3 mg. nitrogen per c.c. Such redissolved precipitates often displayed considerable growth-promoting activity; a completely hypophysectomized rat injected daily with 1 c.c. of one extract gained 28 gm. in twelve days. But the results were inconstant. The filtrates never showed any growth-promoting potency.

Further fractioning of the globulin precipitate (by salting out or dilution) yielded contradictory results. Both the *a* value and the growth-promoting qualities were diminished markedly in both filtrates and precipitates.

Finally, Professor J. B. Collip[4, 5, 6, 7, 8] of Toronto most kindly furnished us with samples of fractions, each of which has a distinct physiologic activity. The growth hormone, which Professor Collip informs us produces active growth in hypophysectomized rats when given in doses of $\frac{1}{64}$ c.c. twice daily, contained no reducing substance. The thyrotropic fraction contained none. The adrenalotropic fraction contained a trace. The maturity hormone contained 1.3*a* units per cubic centimeter—certainly a very small amount.

SUMMARY

1. A method is described for quantitating the reducing power of the tissue of the anterior lobe of the hypophysis.

2. Active reduction may be produced by extracts devoid of hemoglobin.

3. The reducing substance tends to remain with the globulin fraction, which usually also contains the growth-promoting hormone, but the two do not appear to run parallel.

4. Extracts containing the growth, thyrotropic, adrenalotropic, and maturity hormones, respectively, supplied by Dr. Collip, contained no more than traces of reducing substance.

NOTE: After this article was ready for press, our attention was called to an article by Gough and Zilva[8] also describing a reducing substance in the anterior pituitary, which these authors identify with ascorbic acid.

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IMPROVED METHODS IN THE CULTURE OF STERILE MAGGOTS FOR SURGICAL USE*

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THE use of sterile blowfly maggots in the treatment of infected wounds, introduced to surgery by Baer,[1] has grown rapidly during the last two years, and the maggot treatment has now been given in every state in the United States and in several foreign countries. Maggots are usually purchased from certain medical supply houses, but an increasing number of hospitals are now producing their own supply. With the proper equipment and training the private rearing of maggots is quite practicable. The Bureau of Entomology has developed methods by which sterile maggots can be produced on a small scale both conveniently and economically. Some knowledge of the biology of blowflies, as well as familiarity with rearing methods and bacteriologic technic, is essential. Since the former article[3] was written the improvements in cultural technic have been so extensive as to require a revision of the whole process.

SPECIES OF MAGGOTS SAFE TO USE

Both *Lucilia sericata* and *Phormia regina* are satisfactory for cultural purposes and also for use in wounds. *Calliphora erythrocephala* has been found to give less favorable results in the wound and is more difficult to rear throughout the year. Its use was reported in one large series of cases in the United States, but specimens later sent into the Bureau of Entomology for determination were identified as *L. sericata*. These species are all safe for surgical use. There

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is serious danger, however, in the use of screw worms, which belong to the genus Cochliomyia, and they must be carefully avoided as they do not distinguish between dead and living tissues and will penetrate healthy tissue.

BIOLOGY OF FLIES AND MAGGOTS

The eggs, which develop into maggots, are laid by the flies upon meat in colony cages. They are small and whitish and are usually laid in masses. As the eggs are soft, they should be handled carefully. Hatching occurs within twenty-four hours. Eggs are used for two purposes: (1) for propagation of new generations, as flies live only from four to six weeks; (2) for production of sterile maggots to be used in wounds. The eggs for both these purposes are obtained from a common source and no distinction is made until later. Treatment of eggs for propagation is discussed in the next section, and for surgical use in the following one.

Maggots begin to feed shortly after hatching. They partially liquefy their food and take it up in this condition. They are pointed at their head end. When feeding in liquids, they breathe through two spiracles in the heavy, posterior end of the body. Maggots have no eyes, and light is not necessary for their development. Those intended for propagation, called brood maggots, grow rapidly and become full size in from five to seven days. They then leave their food in search of a secluded place to pupate.

After the maggots have migrated, but before pupating, they remain in a prepupal, maggotlike, stage for several hours. In this stage further development is sometimes retarded by placing the specimens in cold storage at from 40° to 45° F. Although this retardation is a convenience, the practice is inadvisable as the Bureau of Entomology[5] has found it to destroy a large percentage of the specimens.

With continued development the prepupa changes into a brownish, oval pupa. This is a quiescent stage, and no feeding takes place. Transformation from the maggot to the fly occurs in this stage and occupies about seven days. Maggots that have been used surgically do not pupate as they are destroyed after removal from the wound.

The female fly normally begins laying eggs from five to ten days after its emergence. The number laid daily varies considerably but averages about 100. The egg-laying period covers from four to six weeks, but the egg-laying capacity of flies and the viability of the eggs begin to decline in three weeks, after which the old colonies should be destroyed and replaced with fresh young ones. Continued inbreeding of the same strain is apparently not detrimental to the progeny.

During autumn and early winter some irregularity in egg-laying may occur. For some days very few eggs may be laid, and it may be supposed that the colonies are degenerating. Resumption of egg-laying, however, usually takes place within a few days. These fluctuations which occur although summer conditions are supplied in the fly cabinet, are regarded as the result of an inherited tendency to cease activity in winter.

While any range of temperature between 70° and 85° F. is tolerated throughout the life cycle, 80° is especially favorable. A uniform temperature is advisable to control the rate of growth and activity. A moderate degree of humidity in the air is desirable in all stages of development, and 50 per cent gives good results. Under these conditions the life cycle of the species is from forty-five to sixty days. A method of supplying heat and humidity is described below.

MAINTENANCE OF REARING STOCK

Incubator or Rearing Cabinet.—As blowflies normally hibernate in winter, the laboratory production of maggots at this time is unnatural. Accordingly additional heat and humidity are supplied in winter to keep the species active. A cabinet for this purpose can be made at small cost. It is constructed of wood with glass doors and with a shelf that allows two layers of cages to be used. The size will, of course, depend upon the number of colony cages used. Inside dimensions of 4 feet in height, 4 feet in width, and 2 feet in depth will be ample for 12 cages.

Electric light bulbs installed on the floor of the cabinet at one end and connected with a thermostat will supply heat conveniently. About six 100-watt bulbs would ordinarily be sufficient. This number will use 6 amperes of current, so a thermostat of sufficient capacity should be used, otherwise a relay should be added. A switch which permits three heats is also advisable.

The humidifier, placed at the other end of the cabinet, consists of a shallow, broad pan of water with a frame to allow lengths of absorbent cloth to hang in the water. A baffle board or false floor, having a number of one-inch holes, is supported above the heater-humidifier to prevent most of the air from rising directing into the cabinet but allowing it to rise mainly around the edges of the board. The part of the floor above the heater is protected by a sheet of asbestos. An opening 4 inches square covered with wire gauze is made in the side of the cabinet by the heater to allow fresh air to enter, and from 4 to 6 one-inch holes are made near the top of both sides for exit. A small electric fan, placed between the heater and the water pan to force the air forward, will give more general circulation and is recommended. The actual degree of humidity in the air is difficult to determine, and most private laboratories will probably have no record of the humidity. The humidifier should then be used continuously, and in winter several thick absorbent cloths should be suspended in the water. With an electric fan fewer cloths are needed. The ordinary dry air of the laboratory in winter is detrimental to development.

In rearing maggots for propagation, when raw meat is used, disagreeable odors will develop. A smaller separate cabinet is advisable, therefore, with an exhaust fan on an outside vent to draw off the odors at the top of the cabinet. Unpleasant odors do not occur in the culture of sterile maggots.

Cages for Fly Colonies.—Flies are kept in colonies, each consisting of about 150 individuals. A simple cage can be made of gauze over a frame of wood, from 10 to 12 inches in each dimension. Upright posts are secured

firmly to the corners of the solid wooden floor and supported at the top with horizontal pieces on all four sides. This is covered on the sides with muslin gauze held in place with thumb tacks. In one of the sides a sleeve of the same material is attached for access to the cage. The top is covered with metal screening so the inside will be visible. This is an inexpensive cage and is easily made.

Another type devised by Simmons[7] is especially rugged and serviceable and permits rapid handling and cleaning. It is more expensive, but it will outlast the other cage. The top has a large circular opening and is braced beneath to prevent cracking. The sides are six metal rods threaded at top and bottom and held in place with two nuts at each end. The cage is 14 inches high and 10 inches in diameter. A cloth sack of muslin lawn is made to be pulled up over the cage and fastened at top and bottom with a cord in a slot cut around the edges. The sack has a short sleeve as described for the other cage, and the top is covered with metal screening, celluloid, or cellophane.

Care of Fly Colonies.—No consideration need be given to the proportion of the sexes. The ratio is usually 1 to 1, and the number of males that occur in the colonies is satisfactory for fertilization.

The food of flies differs greatly from that of maggots. Flies will thrive if a Petri dish of granulated sugar is kept in each cage and supplemented twice a week with Baer's mixture[1] of 70 c.c. water, 30 c.c. honey, one quarter cake of yeast, and one egg. This mixture is best supplied on a pad of absorbent cotton in a separate dish. The remainder will stay fresh only a few days and should be stored in the refrigerator. Flies require water, and this is conveniently supplied by a water fountain described by Miller.[2] It consists of a small beaker of water inverted upon a Petri dish containing one or two sheets of filter paper in the bottom. Flies would drown in water supplied in an open dish.

A piece of lean beef is placed in another dish for the flies to lay eggs upon. For a colony of 150 flies a piece of meat two inches square is ordinarily sufficient. Under the "one-day" method of collecting and sterilizing eggs, described later, a larger piece is used. The meat is given as soon as the flies emerge as it stimulates egg-laying.

No special care is required to provide light for the flies. The ordinary light of the laboratory is sufficient. Exposure of the colonies to direct sunlight or ultraviolet light is unnecessary. Flies are attracted to a bright light, and when cages are opened a light shining through the opposite side will draw them and prevent their escape.

Cages should be kept reasonably clean. While sterility of surgical maggots does not depend upon aseptic conditions in the fly cages, it is advisable to avoid unnecessary contamination of the eggs. When cages become noticeably soiled, flies can be transferred by inverting a clean cage over the soiled one and removing the intervening screen. Transference is hastened by darkening the lower cage.

How to Avoid Dwarfed Flies.—The only function of flies is the production of eggs, and for maximum usefulness flies should be large and vigorous.

Dwarfed flies lay fewer eggs, and their life is much shorter. Several factors can cause dwarfing of flies. The injury is done in the maggot stage; flies cannot change in size. If maggots are forced to migrate from their food prematurely, it is almost impossible to get them to resume feeding after correcting the trouble. They will tend to pupate and eventually emerge as small flies. Premature migration can be caused by: (1) insufficient amount of food for brood maggots; (2) placing excessive numbers of eggs upon the meat and causing overcrowding of maggots; (3) accumulation of gases in the feeding cage, through insufficient ventilation. The fourth cause of dwarfed flies is prolonged cold storage of prepupae. A loss of water which takes place in storage[3] results in both high mortality and stunted flies. Instructions for the proper management of feeding are given in a following section.

Cage for Brood Larvae.—To replace fly colonies before egg production begins to decline, a certain proportion of maggots must be reared through to the fly stage. Cages can be made of glass beakers, dressing jars, or commercial oiled-fiber cans.[4] The latter has several advantages over the others. The cage consists of two containers; an inner one about 3 inches wide to hold the meat, and an outer one from 2 to 3 inches wider and higher for pupation. An inch of sand is placed on the bottom of the outer container. To exclude parasites or other invaders, it is well to cover the outer cage with fine gauze. A heavier material should be avoided as some ventilation is necessary to prevent accumulation of injurious gases.

Care of Brood Larvae.—A larval cage of these dimensions is large enough for the development of about from 250 to 300 maggots. The inner container is three-quarters filled with raw, lean beef such as scraps, hamburger, or other inexpensive pieces. The meat should be moist. Plenty of food must be provided to avoid premature migration as already discussed. Approximately from 200 to 300 eggs, weighing from 20 to 30 mg. are placed down among the meat, and the inner container is left uncovered and put on top of the sand in the outer one. Under favorable conditions the maggots will feed until full grown and then migrate to the sand to pupate. After migration the food residue, which is foul-smelling, should be disposed of at once. Occasionally a few maggots fail to migrate, and they should be discarded with the residue. After migration the larvae can be left in the sand until they pupate, or placed in a beaker with sand in clean colony cages.

Removal of Odors.—Foul odors which develop during production of brood larvae need not become objectionable. The ventilator described for the larval cabinet will remove odors effectively. A household fan, however, used within the cabinet to force air up the pipe is inadequate; and a small exhaust fan in the ventilator line to draw off the odors is practically essential. Residue from brood cages and other waste can be kept conveniently in a paper bag within a large dressing jar or fiber can. This should be stored in the larval cabinet or a chemical hood and burned each day.

First Steps in Maggot Production.—Vigorous colonies of flies are one of the first requisites. Foundation stock to start colonies can usually be obtained from a laboratory already producing maggots. It is best shipped in the

prepupal or the early pupal stages and enough should be requested to start three colonies. Upon arrival from 100 to 150 should be placed at once in each fly cage. If any have not yet pupated, a beaker with an inch of sand should be used. Granulated sugar and the water fountain are added and the cages placed in the rearing cabinet. When adults appear, the meat should be included and renewed often enough to keep it from drying. Flies will begin to lay eggs in from five to ten days.

A large number of maggots should be reserved for the next generation of flies before the production of sterile maggots is attempted. As to the number of colonies to maintain, a convenient index is that each female will lay a daily average of about 100 eggs. A colony of 150 flies will, of course, have only about 75 females.

CULTURE OF STERILE MAGGOTS

The same colonies of flies that produce brood larvae are also used in the culture of surgical maggots. Maggots for use in wounds are cultured under aseptic conditions, and careful bacteriologic technic must be employed. The maggots cannot be sterilized, but sterility can be obtained in the egg stage and the maggots kept from contamination by the use of sterile containers and food.

Removal and Care of Eggs.—The eggs are laid in masses upon the meat, usually in crevices or around the lower margins. Because of their short hatching period all eggs are removed with meat from the fly cages at the end of the day. Egg masses are carefully picked off with a scalpel and placed on a piece of wet cloth laid on a wet cotton pad in a Petri dish. If clumps are large it is best to place them on half the cloth, turning the other half over them. The wet cloth facilitates separation of the eggs described in the next section. The dish is covered and stored in the refrigerator until the next morning when the eggs are separated and sterilized.

Separation of Eggs.—This is necessary before sterilization so the disinfectant can reach the entire surface of each egg. Incomplete separation may result in failure to sterilize. The most satisfactory method tried is the one devised by Simmons.[8] The process includes the moistening of the eggs on the wet cloth already described. This is done to soften the mucoid material around the eggs. In thirty minutes' contact with the wet cloth the eggs can be separated readily by spreading them thinly on the cloth with a spatula. Black cloth is advisable as small clumps needing further separation can be seen. The eggs are then ready for transference to the disinfectant.

Sterilization of Eggs.—Formalin compares favorably with the numerous other disinfectants tested, and in some respects it is the most satisfactory one tried. Immersion of the eggs in a 5 per cent solution of formalin plus 1 per cent sodium hydroxide for five minutes has been found sufficient to produce sterility in routine maggot culture. The sodium hydroxide was added by Simmons to prevent agglutination of the eggs in the disinfectant, a tendency existing in eggs separated by the wet-cloth method.

Eggs are best sterilized in small lots. For private use, from 500 to 600 is a convenient number for a lot and usually as many as needed in a wound; overcrowding the wound frequently leading to trouble.[6] When produced commercially for shipment the number is better increased to 1,000. These small lots are more adaptable to aseptic technic. The number of eggs in a mass can be determined easily by the weight method,[4] approximately 10 eggs (unmoistened) weighing 1 mg. After a few lots have been weighed the number can be estimated by its size.

A test tube of disinfectant is used for each lot of eggs. The separated eggs are lifted from the wet cloth with a spatula or scalpel, placed in the disinfectant, and stirred gently. After five minutes most of the solution is decanted and the eggs are then washed. A convenient apparatus* for washing the eggs consists of a Gooch crucible containing a piece of bandage gauze about 2 in. square and supported in the neck of a wide-mouth specimen bottle. The eggs are poured from the test tube on to the gauze and washed with 100 c.c. of water. A separate unit of this kind is used for each lot of eggs. The crucible and gauze are protected from contamination by covering them with an inverted crystallizing dish which rests upon the shoulders of the bottle, and the unit is, of course, previously sterilized intact. The eggs are transferred upon the gauze directly to the food.

Sterile Maggot Food.—The maggots are reared in glass containers. Shell vials 85 mm. high and 35 mm. wide, or 2-ounce specimen bottles with extra wide mouth and small shoulders, make convenient containers. They are cleaned and plugged with gauze-covered cotton and sterilized. The food is introduced next.

There are now two types of sterile food. The older type makes the maggots grow rapidly and consequently retardation of their growth in the refrigerator is necessary during the sterility tests (described in the next section). The chief objection to this method is that cold storage kills a large percentage of the maggots.[5] Several formulas are in the literature.

The newer type of food permits only a slow rate of growth up to the time of implantation but does not interfere with feeding and rapid growth in the wound. This food, recently devised by Simmons,[9] consists of one part of evaporated milk to from five to seven parts of water, to which is added 1.5 per cent plain agar. The mixture is cooked in a double boiler for 20 minutes, and about 15 c.c. is poured into each container. The advantages of this retarding food are that it eliminates cold storage of maggots during the sterility tests, and it permits holding of surplus maggots in reserve a few days without chilling and consequent mortality. The food is also cheap and easy to prepare; and it permits shipment of sterile maggots long distances without the customary ice packing.

After the food is added the containers are replugged, autoclaved for thirty minutes at 15 pounds pressure, and stored in the refrigerator. When eggs are introduced, the bottles are kept in the fly cabinet for hatching. The cloth is best left in the bottle until implantation.

*Devised by Dr. G. F. White, U. S. Bureau of Entomology.

Test for Sterility of Maggots.—About forty-eight hours after hatching, each lot is tested for sterility. A good method is to test some of the partly liquefied food upon which the maggots are feeding. Both aerobic and anaerobic tests must be made. If the retarding food is used, the maggots can be left at room or incubator temperatures while the bacteriologic cultures are being incubated; otherwise their growth must be restrained in the refrigerator. Any lots found contaminated forty-eight hours later must be discarded. The remainder are suitable for surgical use. By this time the maggots are usually from 4 to 6 mm. in length, a size best suited for use in the wound. Larger maggots are less desirable as their feeding period in the wound is too short. Negative cultures are incubated from four to five days longer, as an extra precaution, after the maggots have been released for clinical use. With some practice in the sterilizing technic, a high percentage of sterile lots should be obtained.

Cold Storage of Surgical Maggots.—When an excess of sterile maggots occurs, a common practice has been to store the surplus in the refrigerator until needed. As this causes a high mortality, it is better when a non-retarding food is used to restrict the supply rather than to depend upon cold storage. If the retarding food is used, cold storage is unnecessary and surplus maggots can be held at room temperature for a few days.

A ONE-DAY METHOD OF OBTAINING AND STERILIZING EGGS

A cultural method has been devised[5] whereby eggs may be sterilized the day they are laid. This is done upon alternate days and leaves each intervening day free for other work. The use of only young flies (up to three weeks of age) is especially advisable. Meat is given upon alternate days and larger pieces than usual (3 in. square) are used. Under these circumstances, flies have laid as many eggs in three hours as ordinarily during a whole day. The meat is placed in the fly cages about 9 A.M. and removed at noon. The eggs are then collected and placed upon the wet cloth. In the early afternoon the eggs are separated, sterilized, and transferred to the food as described under the longer method. As these eggs are newly laid and therefore easily killed during sterilization, it is important to use a disinfectant which will not injure them. The formalin solution as described is especially suitable. The food containers with eggs are then placed in the incubator.

PROBLEMS IN THE USE OF MAGGOTS IN WOUNDS

In the use of living maggots in human wounds, there arise certain problems of a biologic as well as a clinical nature. Some of these questions have been discussed from the biologic standpoint in a circular issued by the Bureau of Entomology.[6] The circular deals with such matters as the inability of maggots to reach deep necrotic areas without surgical assistance; the unfavorable effect upon maggots of accumulated wound secretions; implantation of maggots in the wound; avoidance of overcrowding of maggots; a description of various types of maggot retainers over the wound; why maggots sometimes die in the wound; and why they sometimes escape from the wound.

SUMMARY

The use of sterile blowfly maggots in surgery has become widespread in the last three years, and the private culture of maggots is now being attempted in many hospitals. With the proper equipment and training the production of maggots for private use is quite practicable. Detailed descriptions are given of methods and equipment that have been found satisfactory for this purpose. An outline of the biology of blowflies as involved in the cultural processes is also given.

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A MICROMODIFICATION OF THE FOLIN-WU BLOOD SUGAR METHOD USING PERMANENT STANDARDS*

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THE Folin and Wu[1] blood sugar method has been adapted by various workers[2, 3, 4] to the determination of reducing sugar in minute amounts of blood. Such "micro" modifications have proved reasonably accurate for clinical analysis. There is also the added advantage over the more cumbersome ferricyanide method[5] in that a new set of reagents need not be prepared in shifting from the macro- to the micromethod, since as a rule the latter is used only intermittently in cases in which it is undesirable or difficult to obtain larger amounts of blood. Weaker sugar standards are usually required for the micromodifications and their intermittent use increases the uncertainty inherent in the relatively unstable sugar standards. It would, therefore, be desirable to have at hand permanent colored standards corresponding to definite amounts of sugar when such a modification is employed. These standards would also make the method useful clinically when a colorimeter is not available.

The above considerations led to an attempt to devise permanent standards which would not only be reasonably stable (stability over a period of one year was set up as a minimum requirement) but would also cover a range sufficiently wide to enable one to obtain results without the necessity of repeating the test. Standard colors that apparently fulfill these conditions have been prepared as follows:

Solution A.—Fifty milligrams of Alizarine Blue G.S.† and 10 mg. of Alizarine Blue-Black B.G.† are transferred to a 100 c.c. volumetric flash dissolved in distilled water and diluted to the mark.

Solution B.—A distilled water solution of 20 mg. of Alizarine Cyanone Green B extra† per liter.

From these solutions standards are prepared by measuring into test tubes of uniform bore amounts of each as given in Table I.

TABLE I

SUGAR MG. PER 100 C.C.	SOLUTION A C.C.	SOLUTION B C.C.
60	0.30	9.70
100	0.60	9.40
200	1.40	8.60
300	2.30	7.70
400	3.70	6.30
500	5.90	4.10

*From the Research Institute of Cutaneous Medicine of Philadelphia.
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†From the National Aniline and Chemical Company. It may be found that other dyes will give the same color values, however these were selected because they belong to the anthraquinone series and are among the fastest dyes known.

The values in Table I can be plotted on coordinate paper using values for sugar concentration and solution A as abscissa and ordinate, respectively. From the resulting curve such intermediate values for sugar concentration as may be desired can be picked off and standards prepared accordingly.

Below 200 mg. per 100 c.c. color values spaced 20 mg. apart are readily distinguishable permitting comparison with less than a 10 mg. error. Between 200 and 300 mg. standards 25 mg. apart have been found convenient and above 300 mg., 50 mg. spacings which permit interpolations of 25 mg. are sufficiently close.

These solutions, if placed in tubes (14 mm. bore has been found convenient) of noncorrosive glass which are hermetically sealed, will keep for at least ten months. The range covered will take care of practically all blood sugar values usually encountered if used in conjunction with the modification described below.

METHOD

Reagents.—1. Tungstic Acid Solution: To 40 c.c. of 10 per cent sodium tungstate in a liter flask add about 700 c.c. of water, then with shaking, 40 c.c. of 2/3 N sulphuric acid. Dilute to the mark and mix. A precipitate begins to form in this solution after about two weeks; however, it can still be used as a protein precipitant for about two or three months.

2. Alkaline Copper Tartrate: The original Folin and Wu[1] reagent is used. The less alkaline solution advocated later by Folin[6] is not only more troublesome but, as has been shown by Shaffer and Somogyi,[7] a much more alkaline solution must be used to obtain good recoveries in very dilute sugar solutions.

3. Color Reagent: The complex acid molybdate solution of Folin[6] is used. This solution not only gives a better proportionality of color to sugar content than the older reagent, but the color is considerably stronger for a given amount of sugar which is of distinct advantage when but small amounts of blood are used.

Procedure.—Five cubic centimeters of tungstic acid are placed in a test tube. With a 0.1 c.c. serologic pipette, blood obtained from an ear or finger prick (oxalated blood obtained by vein puncture may of course be used) is added to the solution, rinsing the pipette by drawing the tungstic acid solution into it several times. Stopper with the thumb and shake. The precipitated proteins may now be thrown down in the centrifuge or filtered. If filtered, a 7 cm. filter will be found sufficiently large to take all the mixture and give a filtrate of more than 3 c.c. Two cubic centimeters of this filtrate are placed in a test tube having a bore equal to that of the standard tubes, 2 c.c. of alkaline copper tartrate added, the tube stoppered with cotton and placed in vigorously boiling water for eight minutes. The tube is then cooled for about one minute without shaking, 2 c.c. of the acid molybdate reagent added and the contents mixed by side-to-side shaking. After frothing has ceased (about one minute) the color is compared with the permanent standard colors, giving the sugar content directly without calculation.

If the blood sugar is greater than 500 mg. per 100 c.c., sufficient filtrate is available to repeat the determination upon 1 c.c. plus 1 c.c. of water, the result after comparison of course being multiplied by two.

Comparison of colors is greatly facilitated by the use of a 3-hole wooden block, using either daylight or artificial light transmitted through a frosted glass plate.* Such a block is readily prepared and its use makes color comparison sufficiently easy so that one may readily interpolate between standards.

COMMENT

The above modification of the Folin-Wu blood sugar method presents several advantages which should make it of value clinically: Neither marked tubes nor special tubes are necessary since no dilution of the final colored solution is required and the danger of partial reoxidation of the reduced copper oxide is avoided by stoppering the tube during boiling. The latter expedient has been successfully employed by many workers. Only one micromeasurement is made, that of measuring the blood sample, thus ensuring greater accuracy in the determination and avoiding the use of special pipettes. If the blood is collected outside the laboratory it may be transported in the tube in which the proteins have been precipitated without danger of loss of fermentable substances.

It will be noted that the time of heating has been increased from the usual six minutes to eight minutes. This has been done because it has been found[7] that the longer period is necessary for maximum reduction when dealing with very small amounts of sugar. A ten-minute heating period has been advocated by Shaffer and Somogyi but since their figures indicate that maximum reduction is approached at about eight minutes and to reduce the possibility of sugar destruction by the alkali, the latter figure has been adopted. That

TABLE II

COMPARISON OF FOLIN-WU AND AUTHOR'S MICROMODIFICATION USING PERMANENT COLOR STANDARDS

SAMPLE	HUMAN BLOOD SUGAR—MG. PER 100 C.C.	
	FOLIN-WU	AUTHOR'S MODIFICATION
1	56	60
2	64	60
3	72	70
4	79	80
5	84	80
6	88	90
7	92	90
8	113	110
9	140	150
10	188	175
11	193	200
12	206	200
13	310	300
14	326	325
15	330	325
16	360	350
17	365	375
18	404	400

*The complete equipment including sixteen colored standards may be obtained from the Standard Reagents Company, Philadelphia.

consistent results over a wide range of sugar values can be obtained by this modification is indicated by inspection of Table II which compares the results obtained on the same blood specimen by both the Folin and Wu and this micromodification.

Test tube color comparison is of course not as accurate as good mechanical colorimetry, nevertheless results by either method are probably more accurate than is required for clinical interpretation or treatment.

SUMMARY AND CONCLUSIONS

A "micro" modification of the Folin-Wu blood sugar which employs no special apparatus is described.

For use with this modification a procedure for preparing permanent colored standards covering a wide range of values is described.

The feasibility of the use of such standards is discussed and their accuracy compared with the macromethod and with clinical requirements.

The advantages of this micromethod used in conjunction with permanent standards in clinical practice is indicated.

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A PHOTOELECTRIC DROP RECORDER*

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A COMPLETELY satisfactory drop recorder has not yet been invented, if one may judge from the frequent reports of new devices which appear in the scientific journals. So far three main types of apparatus have been described: (1) the direct contact form, in which the drop makes an electrical contact directly between metallic points; (2) the displacement type, in which the drop displaces a conducting electrolyte which establishes an electrical contact; (3) the mechanical contact type, in which the impact of the drop closes a circuit by some mechanical movement.

Apparatus of the first type is uncertain in its operation because of corrosion and gas formation on the contact points, and of course will not operate at all unless the drop is a fairly good conductor. The second type requires rather delicate apparatus and adjustment, and again gas formation at the electrodes will interfere with good conductivity. The third form will operate quite satisfactorily under certain conditions, but the inertia of the moving contact lowers the rate of response, and the inherent vibration frequency of the moving part may cause chattering and false recording.

The apparatus we wish to describe operates on a principle which has not yet been applied to drop counters, so far as we can learn, although it has been in satisfactory use for many years in similar applications. The falling drop is used simply to interrupt a beam of light actuating a photoelectric cell and the necessary amplifying system. The drop acts as a foreign body in the light beam, is not required to be either opaque or conducting, and has no contact with any part of the apparatus.

Fig. 1 shows the optical system used to obtain a concentrated beam of light that can be interrupted to a great extent by the falling drop. The lamp is an ordinary 3-candlepower tail-light bulb, conveniently fed by from a 6 to 8 volt bell-ringing transformer supplied with 115 volt alternating current. The condensing lens can be made of three ten-cent reading lenses put together, with a combined focal length of about 6 cm. If very small drops are to be recorded, the light beam can be diaphragmed to the sharpest possible focus by a stop, and with a little care the beam can be focussed so sharply that the movement of a single hair will operate the photoelectric system. For the convenience of a rigid mounting, the lamp, lens and photoelectric cell are set up on the same base. The cell is covered with a black paper jacket with a circular hole to admit the light beam to the target, and should be so placed

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This apparatus was demonstrated in operation at the Cincinnati meeting of the American Physiological Society in April, 1933.

that the circle of light beyond the focal point just covers the target of the cell from side to side. As little extraneous light as possible should reach the cell through the opening in its cover so that the drop may cut nearly all the light that reaches the cell.

The pipette is arranged so that the drop forms only 3 or 4 mm. above the focus of the light beam, and falls very little before it interrupts the beam. If the drop is formed too far above the beam, it may be deflected by air currents and cause unsteady operation. The most favorable location for the drop has been found to be just beyond the focal point, but its position can be adjusted by a few trials.

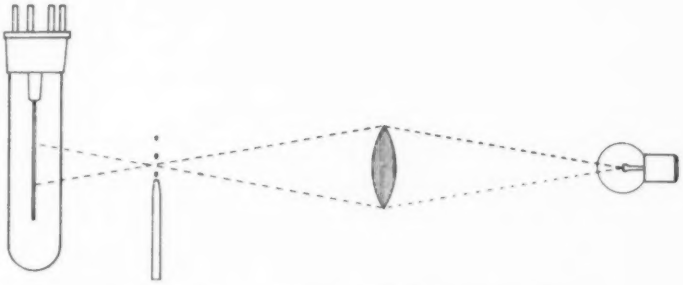

Fig. 1.—The optical system of the drop recorder.

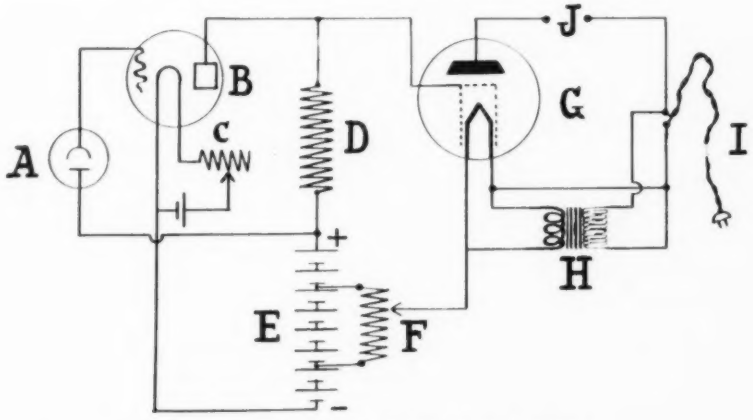

Fig. 2.—The electrical system of the apparatus.

Fig. 2 shows the electrical system employed to amplify the tiny current of the photocell. *A* is the photoelectric cell, which may be either an electron emission cell, or preferably a selenium tube such as the FJ-31 of the General Electric Company. *B* is an amplifying tube of the UX-240 type, supplied with filament current from a 6-volt storage battery through the 20-ohm rheostat *C*. The rheostat may be omitted with only a slight loss in the life of the tube. *D* is the load resistor of the amplifying tube, preferably of 200,000 ohms. *E* is the plate battery of 90 volts, made up of two 45-volt radio B batteries. As the current drain is slight, these batteries will run down very slowly. *G* is the heavy power tube, a G. E. thyratron FG-17. This peculiar

device is a mercury ionization tube which has the advantage of operating on raw 115-volt alternating current, with an output at this voltage of half an ampere. The filament-heating current for the thyratron is supplied at 2.5 volts and 5 amperes from a small step-down transformer *H*, which can be obtained at little cost from a radio supply store. No rheostat is needed in the filament circuit. The output of the thyratron is shown at *J*. The signal marker should be of high resistance with as many turns of wire as possible, since the internal resistance of the thyratron is much higher than that of a battery. *I* represents the electric cord and attachment plug for the supply of operating current from any 115-volt alternating current outlet.

The thyratron fits an ordinary four-pronged radio tube socket, and the connections to the grid and filament are made in the usual way. The plate connection, however, is made to the knob at the top of the tube in order to space the contacts wide for high voltage operation. The output of the tube is half-rectified alternating current.

In operation, the apparatus is plugged in, the light and amplifying tube turned on, and the thyratron is allowed to heat for about four minutes until the contained mercury is volatilized. With the light on the selenium tube, the potentiometer leads are connected to the B battery so that the thyratron can be turned on and off by rotating the potentiometer knob. With the thyratron just turned off, the pipette is placed in position so that the falling drop cuts the beam of light and causes the thyratron to flash on as each drop falls. When well adjusted, the thyratron should turn on for a 10 per cent reduction in light on the selenium tube, or a 30 per cent reduction on a caesium photoelectric cell. The sensitivity can be tested by moving a card into the light circle between the lens and the focal point, and estimating the amount that must be cut off before the thyratron flashes on. The amount of light cut-off required can be varied by turning the knob of the potentiometer. One lead from the potentiometer to the battery should be disconnected when the apparatus is not in use.

The amplifying system of the apparatus can be connected to the photoelectric cell by any reasonable length of wire, so that the selenium cell can be placed in a convenient location for the registration of the drops. The output circuit can be of any length. It is also a simple matter to adapt the apparatus for use as a time signal. The light beam can be interrupted by a swinging pendulum, or a vane on the rotating spindle of an electric clock can be arranged to pass through the beam at regular intervals. Many electric clocks of the synchronous motor type have a shaft which rotates once a minute, and a wheel can be mounted on the shaft with spokes of varying lengths so that time intervals of 10, 15, 20, 30, and 60 seconds can be obtained according to the distance from the center at which the light beam strikes the spokes. If time signals are to be dispensed to a whole class, it is advisable to pass the output of the thyratron into a 250-ohm relay, and send out secondary battery current at about 10 volts to the individual tables. In this way the disastrous effects of a short circuit on the thyratron can be avoided.

A word of caution may not be amiss. The apparatus as described would be classed as a moderately sensitive photoelectric apparatus, and as such demands the care usually given an instrument of precision. The whole set can be assembled in a couple of hours by anyone slightly acquainted with the wiring of radio apparatus. All connections should be cleaned and soldered, and the storage and B batteries should be kept up to their rated voltage at all times. If the voltage of the supply mains varies widely, the light may fluctuate enough to cause false recording. In such a case the lamp must be fed from a storage battery. The thyratron should never be short circuited, since the heavy ionic bombardment caused by a short circuit quickly ruins the filament. The thyratron will operate only on alternating current. On long runs the potentiometer may require slight readjustment as the vacuum of the tube diminishes, but otherwise there is nothing to be adjusted. With these few precautions the apparatus operates with little or no attention.

A MORE CONVENIENT MICROPROJECTION APPARATUS*

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FOR group study of microscopic preparations, whether in classroom or conference, the projection microscope has certain very obvious advantages; it can be used extemporaneously, with the same preparations that have been used for individual study and without the delay incident to photography, objects are seen in their accustomed colors, and by moving the slides about or changing powers the relations of, or variations in different portions of sections or other preparations are clearly and quickly demonstrated. In most institutions, however, the instrument is used but rarely. Aside from difficulties rising from flicker or inadequacy of the light source, much of the disappointment in it has seemed to be due to the experience that its use involves much lost motion and is time consuming. In the usual apparatus, slides must be picked up and slipped in a vertical position beneath clips or into a mechanical stage. They are moved about in an unfamiliar manner, and altogether the demonstration lags far behind the natural rate of the accompanying discussion.

To overcome this objection, the apparatus to be described was devised and has been used with much satisfaction in pathologic conferences. Ideally, such an apparatus should: (1) Provide for rapid, easy, and natural manipulation and interchange of slides. (2) Have a considerable range of magnification, changed with a minimum of time and effort. (3) Give approximately "critical illumination," with as few motions on the part of the operator and as near an approach to a positive setting for the more used powers as can be arranged. Most projection microscopes have been adaptations in mounting of more or less specialized variants of the familiar microscope stand. Our thought

*From the Jeanes Hospital.
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has been, on the contrary, in order to arrange for the first of the above desiderata, to start with the table top and build the microscope into it, making the table serve as the microscope stage. The microscope tube is kept in a vertical position by the familiar expedient of reflection of the light source and of the projected image. With this arrangement, a considerable number of slides may be interchanged purely by a sliding motion. The slide being demonstrated is moved exactly as when the microscope is used individually and

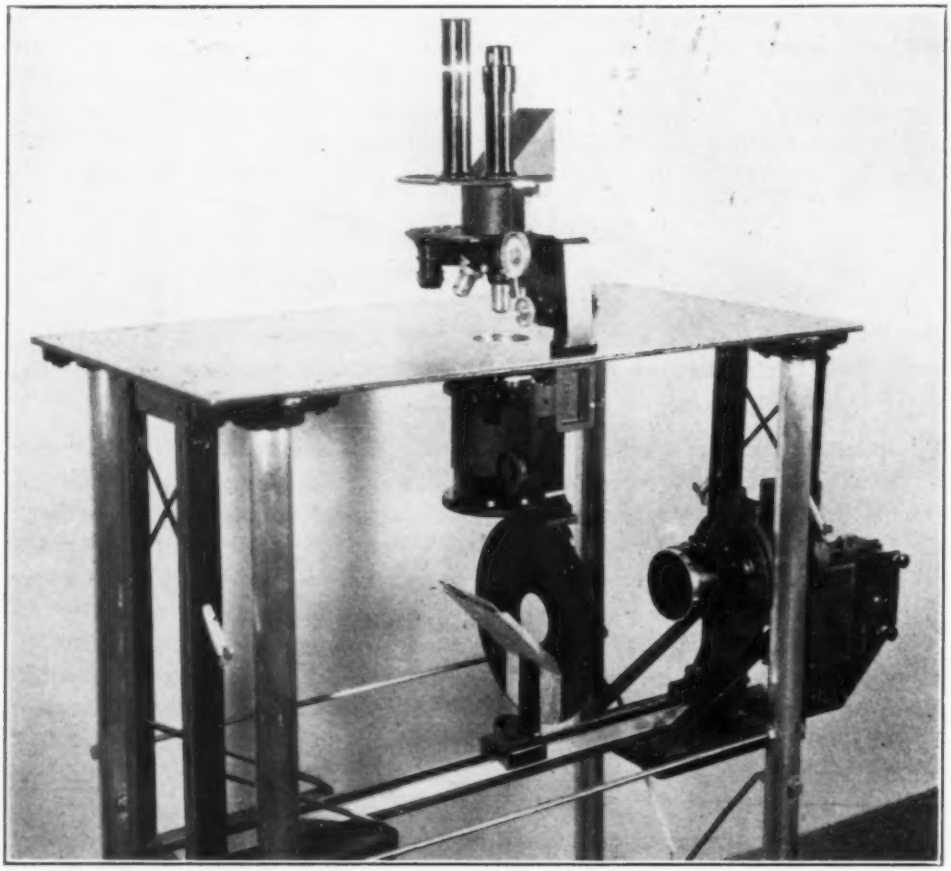

Fig. 1

gravity is an aid instead of a hindrance to the holding of a field once selected. In fact, clips are not desirable under most conditions, though holes have been provided for them and for a mechanical stage.

It has been constructed by taking apart a Bausch and Lomb "Large Projection Microscope" sufficiently to remove the stage. Holes were bored in a steel plate measuring 25 × 15 inches, and tapped and provided with pins exactly to correspond with those in the original stage. The microscope was then reassembled, with the steel plate taking the place of the stage and serving as the support of the arms carrying the microscope above and the substage

below. The central opening was made 1¾ inches in diameter, countersunk on top to provide for a blackened brass disk with a one-inch opening. Brass legs 42 inches long and suitably braced were attached to the four corners. Entirely out of contact with these legs is a support for a metal optical bed, suspended from the ends of the steel plate 15 inches below it. This is made rigid by diagonal bracing, and provision is made for horizontal adjustment at a right angle to its length by means of wing nuts on the braces across the ends. Since the end supports have been made to form an exact parallelogram, the surface of the optical bed remains parallel with the steel plate during any such change. An automatic feed arc lamp is attached to the optical bed at one end. With an 8 ampere rheostat and 5/16 inch cored carbons this is nearly free from flicker and wandering of the crater. Under suitable conditions, other light sources would probably prove satisfactory and be more convenient than the arc. A 45 degree mirror is supported on the optical bed below the axis of the microscope.

The substage, attached below the steel plate, carries a large condenser for use with microtessars or similar lenses, and above this two smaller condensers of differing numerical apertures on a swinging arm for use with microscope objectives. A recess must be drilled partially through the plate for the small condenser not in use, to permit the condenser in use to be elevated close to the slide for objectives of high aperture.

The stand has a 5½ inch revolving plate which carries a quadruple nose piece and openings for three low-power objectives and microtessars. Another similar plate has two tubes for eyepieces and a 45 degree first surface mirror, the latter for low-power projection without an eyepiece. A prism is used above the eyepieces to project the image in a horizontal plane; it can be turned in any direction. Since the stand was intended for use in another manner, the mirror is mounted to throw the beam of light "backward" across the supporting arm. The operator is thus obliged to stand in an inconvenient position. By filing away a portion of a corner of its support, and sacrificing one of the eyepiece tubes, the mirror can be reversed and the operator can then be in a natural position behind the stand. (This was not yet done when the accompanying photograph was made.)

With casters on the legs, the prism is about 54 inches above the floor. When further elevation of the projected image is desired, the entire apparatus is tipped backward slightly. With small groups this is not often necessary. Hinging of the top would provide for elevation with greater stability, but it is very desirable to avoid cross structural elements which would interfere with easy manipulation of the substage.

Thanks are due to Mr. J. I. Wexlin, local representative of Bausch and Lomb, for suggestions regarding light sources and suitable optics for projection.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MALARIA, Blood Groups and Therapeutic, Polayes, S. H., and Derby, I. M. J. A. M. A. 102: 1126, 1934.

A series of 127 cases of dementia paralytica treated with malarial blood was studied in order to determine (a) the effect of the injection of compatible or incompatible blood on the incubation period, (b) the occurrence of immediate or delayed reactions to incompatible malarial blood, and (c) the frequency of occurrence of so-called primary fever. The following conclusions may be drawn:

1. The average incubation period is 4.30 days when the malarial blood injected is compatible and 8.18 days when it is incompatible with the recipient's serum. That this difference is significant was proved by statistical analysis.

Wide variations of the incubation periods were noted in the group of patients receiving compatible as well as in those receiving incompatible blood. These differences may be ascribed to factors other than compatibility or incompatibility of blood.

2. With regard to untoward postinoculation reactions, it was not possible to determine with any degree of certainty that they were due to incompatibility between the donor's and the recipient's blood. Other possible factors may be responsible for these reactions.

3. Primary fever occurred in only 15 per cent of the patients receiving compatible malarial blood. These figures are much smaller than those found by previous investigators. The difference is perhaps due to the fact that it is difficult to recognize this phenomenon because the postinoculation temperature curve is frequently too irregular to conform rigidly to the definition of primary fever.

B. C. G., Spontaneous Occurrence of a Non-Acid-Fast Form in a Culture of, Sidenberg, S. S., and Ecker, E. E. Am. Rev. Tubere. 29: 571, 1934.

A nonacid-fast variant of B. C. G. was discovered in a culture obtained from Paris on February 4, 1932.

The variant possessed the growth properties of the tubercle bacillus and reverted to the acid-fast form when grown on various tubercle bacillus media over a sufficient lapse of time.

The variant showed none of the characteristics of a contaminating organism.

Guinea pig inoculations yielded cold abscesses at the site of the injection. Acid-fast organisms were recovered from these abscesses.

Although the B. C. G. is not a fixed form of microorganism and though it may yield a variant as described, the variant was devoid of pathogenicity for guinea pigs.

B. TYPHOSUS, Evidence That B. alkalescens (Andrewes) May Be a Variant of, Gilbert, R., and Coleman, M. B. Am. J. Pub. Health 24: 449, 1934.

It has been shown that strains of bacteria having the properties of *B. alkalescens* are present in approximately 1 per cent of all specimens submitted for bacteriologic examination for evidence of enteric disease. Clinical and epidemiologic data suggest a close relationship to the typhoid bacillus.

In the study of specimens from patients recovering from typhoid fever and in the search for carriers, the presence of this type of microorganism seems of special import. Even though, in itself, it may have no pathogenic significance, the fact that in so many instances *B. typhosus* has been isolated from the same or other specimens from the individual concerned, makes a thorough search for this microorganism imperative, whenever strains of *B.*

alkalescens are found. When suspected typhoid carriers are concerned, it would seem very important to examine a large series of fecal specimens and, if possible, duodenal contents.

PYURIA, Frequency of, in Anomalies of the Urinary Tract in Children, Begler, J. A. Am. J. Dis. Child. **47**: 780, 1934.

Anomalies of the urinary tract are of frequent occurrence, and the majority of them are congenital. In this series, so far as could be determined, none of the urinary obstructive lesions were due to acquired stricture caused by infection or inflammatory reaction in the ureteral wall.

Many such lesions go unrecognized for a number of years because of the lack of recognizable symptoms.

Obstructive lesions with urinary stasis may be present for years without infection and pyuria.

Since 50 per cent of the children with urinary stasis had pyuria, it appears that urinary infection in these children may be more frequent than in normal children, but that stasis, contrary to the usual belief, does not always lead to pyuria. When infection does take place it is exceedingly persistent, even becoming permanent, unless the obstruction is relieved.

Catheterization or the manipulations of urologic investigation invariably lead to infection when stasis is present.

A normal urine does not exclude the possibility of an obstructive lesion of the urinary tract.

ENTERITIS, Relationship of Shigella Alkalescens to Other Members of the Shigella Group, Welch, H., and Mickle, F. L. Am. J. Pub. Health **24**: 219, 1934.

Cases of dysentery-like diseases and others apparently caused by *Shigella alkalescens* are reported.

The relationship of *Shigella alkalescens* to other members of the dysentery group is demonstrated by means of agglutination, agglutinin absorption and the Schwartzman phenomenon.

Shiga antifiltrate horse serum neutralizes in rabbits and mice the toxic effect of *Shigella alkalescens* filtrates.

This investigation indicates that *Shigella alkalescens* should be included among those organisms in the Shigella group capable of causing disease in man.

FOOD POISONING, Suggested Laboratory Procedures for Use in Determining the Cause of, Koser, S. A. Am. J. Pub. Health **24**: 203, 1934.

A. First Examination:

1. Prepare a stained smear (Gram stain) directly from the foodstuff, preferably the liquid portion.

2. With a loopful of the foodstuff streak in succession 2 agar plates, preferably a meat infusion agar. With another loopful streak 2 more plates of either Endo or eosin-methylene blue agar. Incubate for from eighteen to twenty-four hours at 37° C.

NOTE: If the only sample submitted is an empty jar or can, the interior should be thoroughly washed out with a few c.c. of sterile salt solution or sterile broth. The washings can then be used for the examination.

3. Inoculate a tube of broth, preferably prepared from meat infusion, with a small amount of the sample. Incubate at 37° C.

If the poisoning is suspected or definitely known to be botulism the following steps should be included:

4. Inoculate each of 3 tubes of ground meat or beef heart medium with approximately a gram or 1 c.c. of the sample. Ground lean beef, beef heart or veal is tubed with the infusion from the meat so that the layer of ground meat occupies about half of the column of the liquid. The final pH should be from 7.2 to 7.6.

Immediately after inoculation 2 of the 3 tubes should be heated to 80° C. for twenty minutes to destroy vegetative cells. If an anerobic jar is not available the 3 tubes should be layered with about 1 c.c. of sterile vaseline to form a seal. Incubation should be at 35° to 37° C.

5. A portion of the sample should also be used to test for the presence of toxin. Inject subcutaneously at least 2 guinea pigs or 2 white mice with a portion of the food. In most cases it will be necessary to centrifuge the sample before injection to get rid of gross contamination. Use 0.5 to 1.0 c.c. of the supernatant fluid for injection. Where abundant toxin formation is suspected and sufficiently large sample is available, it is desirable to feed portions to 2 guinea pigs.

Should death of any of the animals occur, controls with antitoxin of types A and B should be included. Inject subcutaneously 2 more animals with a protective dose of Type A antitoxin (usually 1 c.c. is sufficient) and an additional 2 with Type B antitoxin. These 4 animals should then be injected with the foodstuff in the same way as the original 2 which did not receive antitoxin. If the antitoxic sera are not at hand, these controls should be performed as soon as they are available.

B. Subsequent Examinations:

1. Microscopic examination of the stained smear will give an idea of the relative abundance of bacteria in the product and of the morphologic types present.

2. Examine the Endo or eosin-methylene blue plates for the presence of colonies resembling those of the paratyphoid, typhoid, or dysentery groups. If suspicious looking colonies are found, fish several to either Russell medium or to fermentation tubes of dextrose, lactose, and sucrose broths. If such colonies are not encountered, the plates should be incubated an additional twenty-four hours and reexamined. The identification of suspected intestinal pathogens requires additional procedure and is described in Section C.

Examine also the nutrient agar plates and make Gram stains of several representative colonies. Note especially if staphylococcus or streptococcus colonies are present in considerable numbers.

3. The purpose of using a tube of broth is to detect by enrichment and subsequent plating any Salmonella, staphylococci, or other types which might have been missed by direct plating. If the steps listed under A2 and B2 have yielded no information of value, streak Endo or eosin-methylene blue plates and nutrient agar plates from this broth culture. The examination of these plates should then proceed as given under item B2.

4 and 5. (If suspected botulism.) The condition of the animals which were injected under item A5 will afford preliminary evidence of the presence or absence of botulinum toxin in the food. This should not be supplemented by examination of the meat medium tubes inoculated under item A4.

These tubes should be incubated for at least three or four days. Note any macroscopic evidence of growth and prepare Gram stains from each tube. Note whether gram-positive bacilli, with or without subterminal spores, are present. Select one or more tubes for a toxicity test similar to that carried out with the original sample under item A5. The result of this will serve to confirm that secured with the food sample and at times it may be of

RUSSELL'S MEDIUM (24-HOUR READING)	FERMENTATION TUBES			
	DEXTROSE	LACTOSE	SUCROSE	
Acid and gas in butt, alkaline slant	Acid and gas	Negative*	Negative*	Similar to that produced by Salmonella group
Acid in butt	Acid	Negative	Negative (or occasionally acid)	Similar to Flexner dysentery
Acid in butt	Acid	Slow acid	Slow acid	Similar to Sonne dysentery

*Incubation should be continued for at least a week or preferably two weeks to rule out delayed fermentation of these sugars. Delayed fermentation of lactose or sucrose is not typical of the Salmonella group.

great value in affording some additional information. In the event of a positive test the meat medium may be used for further purification and isolation of the culture, if this is desired.

C. Checking of Suspected Salmonella or Other Intestinal Types:

Fermentation results similar to those shown in the accompanying outline immediately raise the question whether the organism may be a member of the Salmonella group or one of the dysentery bacilli. Since there are many miscellaneous saprophytes which resemble the intestinal pathogens in these superficial aspects, a somewhat detailed procedure of checking is necessary. When a culture has been selected for further examination a Gram stain should be made to see whether one is dealing with a medium-size, gram-negative, nonsporulating rod.

GRANULOCYTOPENIA, Primary, The Etiology of (Agranulocytic Angina), Madison, F. W., and Squier, T. L. J. A. M. A. **102**: 755, 1934.

The increase in incidence of primary granulocytopenia (agranulocytic angina) has paralleled the increase in the use of drugs containing amidopyrine and especially those containing amidopyrine with a barbiturate.

The disease has appeared most frequently in persons apt to be taking drugs: physicians, nurses, or those directly under the care of a physician.

In each of fourteen patients the onset of primary granulocytopenia was directly preceded by the use of amidopyrine alone or in combination with a barbiturate.

The mortality in a group of six patients who continued the use of drugs containing amidopyrine was 100 per cent. In a group of eight patients who did not continue the use of these drugs, only two died, and both of these died in the initial attack.

The administration of a single dose of amidopyrine to each of two patients who had recovered from the acute disease was followed by a rapid profound fall in granulocytes.

One rabbit given allylisopropylbarbituric acid with amidopyrine (allonal) by mouth in relatively large doses showed an abrupt drop in granulocytes and died on the thirtieth day. Preceding death there was complete absence of granulocytes in the peripheral blood. Seventeen other rabbits given allonal or amidopyrine showed no significant change in the blood picture.

The authors believe that amidopyrine alone or in combination with a barbiturate is capable of producing primary granulocytopenia in certain individuals who have developed sensitivity to the drug.

They also believe that the appearance of primary granulocytopenia following the use of such drugs may be the result of an allergic or anaphylactoid drug reaction.

B. PERTUSSIS, Cough Plate Examinations for, Kendrick, P., and Eldering, G. Am. J. Pub. Health **24**: 309, 1934.

Cough plate diagnostic service for pertussis has been available continuously for about a year at the Western Michigan Division Laboratory of the Michigan Department of Health, Grand Rapids. It is proving a practicable procedure under the conditions existing there.

During the past four months of this service 23 per cent of the positive diagnoses have been made within forty-eight hours after the plates have reached the laboratory, 75 per cent within seventy-two hours, and 91 per cent within four days.

The laboratory findings are being used by the City Health Department in obtaining earlier diagnosis of pertussis and thereby, it is hoped, more effective isolation of cases in their most infective stage. The possible applicability of the results to the problem of release is under study.

In agreement with the reports of most other authors, *B. pertussis* could be isolated from relatively few patients after the fourth week of disease. A patient with whooping cough of more than four weeks' duration, who can be shown to harbor *B. pertussis*, is defined tentatively as a post-whooping cough or convalescent carrier.

One hundred thirty-six cultures isolated from whooping cough patients, at times ranging from before onset to the thirty-fifth day of the disease, were found to fall into the same serologic group.

ULCER, Oral Administration of Metaphen in the Treatment of Gastric and Duodenal, Trippe, C. M. Ann. Med. 6: 901, 1933.

Metaphen, in 1:500 solution, was given orally to patients with symptoms of chronic abdominal distress in the dose of 4 c.c. three times a day with very gratifying results.

The material presented included an analysis of 82 cases, 26 of gastric and 56 of duodenal ulcers. Complete x-ray studies were made of 27 of these cases; diagnosis was also confirmed in many cases by test meal and microscopic study of gastric contents.

Relief from pain was obtained in practically all cases in an average of three days' time.

No toxic effects were ever observed.

Complete disappearance of gastric and duodenal ulcers consequent upon treatment with metaphen has been demonstrated by means of x-ray studies, made before and after treatment.

A possible explanation of the action of metaphen, primarily a bactericidal agent, may lie in the part played by infection in the evolution of ulcers of the type considered, as has been recently emphasized by several authors.

SPORE STAIN, Modification of Dorner's, Snyder, M. A. Stain Techn. 9: 71, 1934.

(1) Make a thin film of the organisms on a slide. (2) Cover with a small piece of blotting paper and apply several drops of freshly filtered Ziehl's carbol fuchsin. (3) Allow preparation to steam five to ten minutes on a hot plate, keeping the blotting paper moist with carbol fuchsin. (4) Decolorize instantaneously with 95 per cent alcohol and wash with water. (This step may be omitted if the spores do not hold the color well, but in general a neater looking preparation is obtained with its use.) (5) Apply a drop of saturated aqueous nigrosin and spread evenly and thinly over the smear. Allow to dry quickly on the hot plate.

The results obtained (spores red and the vegetative parts of the cells almost colorless) are very similar to those of the unmodified Dorner technic. The procedure is simpler and quicker and is more successful in many cases. The above technic gave good results with spore-formers identified as the following: *Bacillus megatherium*, *B. niger*, *B. cereus*, *B. mycoides*, and with some cultures of *B. subtilis* (Marburg strain). The unmodified Dorner method is quite unsatisfactory for the last named organism; this modified technic is better, but does not give uniformly good results with all strains.

FOCAL INFECTION, Necessity for Revising the Common Conception of, Solis-Cohen, M. J. A. M. A. 102: 1128, 1934.

The common conception of focal infection concentrates attention on the infected tissue rather than on the infecting bacteria.

When the physician speaks of eradicating a focus of infection, what he really means is removing infected tissue, such as tonsils or teeth, or draining and cleaning out an infected cavity, such as a nasal accessory sinus.

Infecting bacteria seldom are confined to a circumscribed area of diseased tissue but are present also on adjacent tissues, which may be apparently free from disease.

Enucleating of diseased tonsils does not necessarily remove the focus of infection, because the infecting organisms may still remain in tonsillar stumps, in recurring tonsillar tissue, in infratonsillar lymphoid tissue, and on the apparently normal mucous membrane of the tonsillar fossae and the nasopharynx.

A focus of infection in a nasal accessory sinus is not necessarily eliminated by treatment or operation, because infecting bacteria may still persist there.

Such expressions as "removal" or "elimination" or "eradication" of the focus of infection should be avoided when one is describing tonsillectomy or sinus treatment or operation.

The conception of focal infection should stress the bacterial element and regard the causal germ as the chief infecting agent.

Surgery alone cannot possibly remove all the infecting organisms, which can be overcome only by the patient's defensive forces.

The elimination of a focus of infection requires the production of bactericidins and other antibodies to destroy the infecting bacteria and render their toxins harmless, which can be aided by hygienic measures but in many cases needs the artificial stimulation of a potent vaccine containing the proper antigens obtained from the infecting bacteria.

The pathogen-selective culture selects the etiologically important organism from a mixed culture, and the vaccine made from it contains both the endotoxins and the soluble exotoxins of the infecting bacteria.

When, in addition to the extirpation of accessible and removable diseased tissue that has become a favorable soil for the propagation of the infecting germs, the required specific antibodies have been produced in sufficient quantities to overcome the infecting organisms and their toxins, the focus of infection can truly be said to be eradicated at both the primary and the secondary foci.

TUBERCULOSIS, An "Acid-Fast" Other Than Koch's Bacillus Cultivated From Sputum, Cummins, S. L., and Williams, E. M. Tubercle, London **15**: 49, 1933.

The patient was a woman suffering from acute pulmonary disease, which had come on shortly after a confinement. The picture was that of acute phthisis, but the sputum had been reported negative on several occasions. The authors found in a sample of sputum numerous acid-fast bacilli, scarcely distinguishable from tubercle bacilli but showing a few curious "balls" of curved rods, which were sufficiently peculiar to raise the question of an unusual morphologic type. From a culture a growth was obtained and from another sample of the sputum sent to Griffith six weeks later the same organism was cultivated. Whether the germ was merely an adventitious saprophyte or the causative organism of the severe pulmonary disease from which she suffered must remain open to question, as opportunities for further biologic tests were refused. The authors emphasize the fact that the finding of acid-fast bacilli in the sputum is not necessarily positive proof of tuberculosis. The importance of cultural verification in all doubtful cases cannot be stressed too much.

HEMOPHILIA, Negative Effect of Prolonged Administration of Ovarian Substances in, Stetson, R. P., Forkner, C. E., Chew, W. B., and Rich, M. L. J. A. M. A. **102**: 1122, 1934.

The coagulation time of the blood of seven patients with hemophilia was observed repeatedly during treatment with ovarian substance or estrogenic substance. Large amounts of one or another of eight different preparations were given over periods of from twenty-eight to eighty-one days. In no instance was the coagulation time of the venous blood found to be depressed as the result of such therapy. Theelin (estrogenic substance) administered subcutaneously to two patients for from three to seven days neither stopped bleeding nor reduced the clotting time.

Estrogenic substance was demonstrated consistently in the urines of these patients both under ovarian therapy and during control periods. No correlation could be established between the quantity of estrogenic substance excreted in the urine and the fluctuations of the blood coagulation time.

No clinical improvement was noted which could be attributed to ovarian therapy. In four of the patients hemarthroses developed without obvious trauma or infection late in the course of treatment.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The International Medical Annual*

WHILE it is true that the progress of medicine is reflected in the current medical literature and that the articles of today are often, as it were, the textbooks of the future, it is also true that few are either in a position or have the time to follow the enormous mass of medical publications which fill the journals every year.

This was the genesis of the International Medical Annual, now in its fifty-second year, which has for its purpose the presentation of a conspectus of current medical literature.

The purpose of these volumes is too familiar, and the issues each year too well known to require extended comment. The present issue maintains the standards set by its predecessors.

The plan of the work, which is, as it were, a dictionary of practical medicine, when coupled, as it is, with a comprehensive index, enables rapid reference to the contents; what cannot be found readily in the body of the text is easily located in the index so that the practitioner in search of specific information may find it quickly accessible.

While the book is primarily a review and survey of the literature of the year, and hence limited as to content to what has or has not been published, many special reviews and articles are included independently of the literature.

The annual can be commended as a comprehensive survey presented in a readable and usable manner.

Pathogenic Microorganisms†

THIS book needs no introduction, having long been an outstanding standard text.

As heretofore the volume is divided into three main sections: Part I concerned with the general characteristics and methods for the study of pathogenic microorganisms; Part II discussing the pathogenic microorganisms individually; and Part III ("Applied Microbiology"), presenting methods for the examination of water, air, soil, shellfish, milk, and a practical discussion of disinfection and sterilization.

This tenth edition represents a thorough and comprehensive revision bringing the volume in accord with all the recent developments in this field.

Among the changes are a revision of the chapter on microscopic technic; a discussion of the newer types of pneumococci and the newer studies of the colon-typhoid group; a discussion of the present status of B. C. G. vaccination; recent studies in bacterial metabolism; recent studies in the preparation of diphtheria toxin and an evaluation of the flocculation test for the determination of its antigenic value; a revision of the section on spirochetes and of the section on filtrable viruses; newer studies in the examination of milk, water, and shellfish, and a revision of the chapter on hypersensitiveness.

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*The International Medical Annual, A Year Book of Treatment and Practitioner's Index. Edited by H. Letheby Tidy and A. Rendle Short. Cloth, 579 pages, 69 plates, 101 drawings. William Wood and Co., Baltimore.

†Pathogenic Microorganisms. By William Hallock Park, M.D., Professor of Bacteriology and Hygiene, University and Bellevue Hospital Medical College, and Anna Wessels Williams, M.D., Assistant Director, Bureau of Laboratories, Department of Health, New York City. Cloth, Ed. 10, pp. 867, 215 engravings, 11 color plates. Lea and Febiger, Philadelphia.

Included, also, are discussions of the practical experience of the authors with active immunization against diphtheria and scarlet fever and the results of recent studies in yellow fever, undulant fever, and poliomyelitis.

The familiar summary table has been extensively revised. This is such a useful form of rapid reference that one wishes it were in sections which could be removed, mounted, and made readily available in the laboratory.

This book, like its predecessors, may be recommended in the highest terms and without qualification as one of the most valuable texts in this field, not only for the laboratory worker but for all who are interested in medicine and allied subjects. Certainly no hospital, board of health, or clinical laboratory can afford to be without it.

An Introduction to Practical Bacteriology*

THIS, the fourth edition of a well-known laboratory manual, has been completely revised to include recent developments in the field of bacteriology.

The volume retains its original purpose as primarily a guide to the practical laboratory study of bacteriology and consists of three main sections. Part I is introductory and in a brief and general way discusses the biology of bacteria and immunity in its relation to practical bacteriology; Part II is devoted entirely to bacteriologic and serologic technic; while Part III presents a description of pathogenic and commensal organisms, as well as the methods applicable to the bacteriologic diagnosis of various infections.

While intended primarily for the student the volume may well be considered a useful reference work for the physician and laboratory worker in general. As is commonly true of European texts, while the American literature has not been neglected, the references are predominantly European in origin. This does not, however, detract from the general usefulness of the work.

This book may be recommended to all who are interested not only in bacteriology per se but also in its practical application to the study of disease.

Treatment of the Commoner Diseases†

THIS book is based upon ten lectures constituting a postgraduate course of instruction and, as the title shows, is concerned with those disorders commonly encountered in general practice.

Although in this age of specialism the fact appears at times to be somewhat lost sight of, it is nevertheless true that no specialty demands the comprehensive knowledge and ability essential to the efficient conduct of a general practice. For the general practitioner, to be truly efficient, should be, first of all a specialist in diagnosis.

Few men have had a more comprehensive experience or are better fitted to write such a volume as this than Professor Barker, whose task, as outlined in the preface, has been well performed.

The volume is in no sense a formulary. Very wisely, Professor Barker, emphasizing its essential importance, had discussed the signs, symptoms, and diagnosis of the commoner diseases, embodying a comprehensive survey of the important and useful contributions which have appeared in the recent literature.

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*An Introduction to Practical Bacteriology. By I. J. Mackie, M.D., Professor of Bacteriology, University of Edinburgh and J. E. Mc Cartney, M.D., Director, Research and Pathological Services, London County Council. Ed. 4, cloth, 504 pages. William Wood & Co., Baltimore.

†Treatment of the Commoner Diseases. By Lewellys F. Barker, Professor Emeritus of Medicine, Johns Hopkins University. Cloth, pp. 319. J. P. Lippincott Co., Philadelphia, Pa.

Throughout the volume there appears, of course, the therapeutic experience garnered from his own years of practice but the main purpose of the volume is to afford the practitioner an opportunity to become familiar with recent advances and to suggest sources for collateral reading in those subjects in which his particular interest may be centered.

The book may be recommended as comprising in small bulk a wealth of information clearly put and readily applicable to the problems of the practitioner at large.

Brucella Infections in Animals and Man*

THAT the disease once known as "Mediterranean fever," the cause of which was first described by Bruce in 1886, Bang's "abortion disease" in cattle, the cause of which was first described by Bang in 1897, and the very similar disease in sows first described by Traum in 1914, are all the result of infection with a single bacterial species, the Brucella, and that all three of these organisms are the cause of the disease in man known as undulant fever, is now a matter of common knowledge.

Undulant fever, moreover, as has been shown by the work of many investigators, is now recognized as a common and widespread infection which, because of its chronicity and manifestations, is of great importance.

The present manual, concerned principally with the methods of laboratory diagnosis applicable to its recognition, is a most timely publication which should be gratefully received by both laboratory worker and physician at large.

The author, Dr. Huddleson, has long been known for his extensive and varied studies in this field so that the text may be regarded as authoritative and based upon first-hand knowledge.

The idea of the work is to present the fundamental information concerning the disease and laboratory methods found satisfactory for its study, and to discuss these in such a fashion that the laboratory worker will be able to utilize them and the clinician will be able to interpret their results.

That this objective has been achieved the book itself is evidence.

The thoroughness with which the subject has been covered is shown by the appended chapter headings: The Genus Brucella; Methods of Isolating Brucella; The Pathology of Brucella Infections; Serological Methods of Determining Brucella Infections; Allergic Methods of Determining Brucella Infections; Method of Determining Brucella Infections by Measuring the Opsono-Cytophagic Power of the Blood; and Methods of Differentiating the Species of the Genus Brucella.

A bibliographical as well as general index add to the value of the book which will, without doubt, become the standard reference in connection with this disease.

Recent Advances in Medicine†

THIS, the seventh edition of this well-known book, has undergone extensive revision with the resultant addition of over one hundred pages of new material and seven new figures.

As before, the purpose of the book is to correlate the more recent advances in medicine as concerns the clinical and laboratory investigation of disease and, also, to a somewhat lesser extent, in the field of therapeutics.

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*Brucella Infections in Animals and Man: Methods of Laboratory Diagnosis. By I. Forrest Huddleson, Dept. of Bacteriology and Hygiene, Michigan State College. Cloth, pp. 108, 24 figures. The Commonwealth Press, New York.

†Recent Advances in Medicine, Clinical, Laboratory, and Therapeutic. By G. E. Beaumont, M.D., Physician, Out-Patient Department Middlesex Hospital, etc., and E. C. Dodds, M. V. O., Professor of Biochemistry, University of London. Ed. 7, cloth, pp. 485, 59 illustrations. P. Blakiston's Son & Co., Philadelphia, Pa.

The general manner of presentation remains the same; first, the description in some detail of the methods of investigation applicable to the formation of a diagnosis; second, a discussion of their significance and interpretation; and finally, some discussion of treatment.

Wherever a particular procedure is referred to, its technic is described in sufficient detail to enable the reader to attempt it although, as is unavoidable, for many of the procedures the clinician in general will have neither the equipment, the skill, nor the skilled help necessary.

Nevertheless, the value of any method of study, laboratory or otherwise, is enhanced by an understanding of the principles upon which it is based and of the various factors which may influence or interfere with its results, and, where the physician maintains his own laboratory, the book will be found a safe technical guide.

The needs of the clinician are kept in mind throughout the volume as witnessed, for example, by the careful discussion and tabulation of the high and low calcium diets, ketogenic diets, and the various dietetic schedules applicable to the treatment of diabetes.

It is questionable, however, whether the space given to the sanocrysin treatment of tuberculosis will be justified by the final status of this drug.

The volume well serves the purpose in view of furnishing to the student and clinician a useful reference work embodying the more recent advances in the field of general medicine.

This book may be recommended to the physician at large.

The Chemistry of the Hormones*

THE underlying purpose and intent of this book is to furnish for the student a connected account of the chemical characteristics of the hormones, so far as these are at present understood and to present to the laboratory worker desirous of preparing active hormone fractions or who wishes to isolate a chemically pure hormone, detailed descriptions of the methods available.

The book is thus eminently practical both in concept and in the execution of the plan in view and within its nine Chapters can be said to present in a clear and satisfactory manner the present concepts of the chemistry of hormones.

The subjects covered are: the thyroid hormone; the parathyroid hormone; insulin; the pituitary hormones; the adrenal hormones; the male hormone, the female hormones; secretin; and plant hormones.

While, as indicated by the title, the volume is primarily concerned with the chemistry of hormones their physiological and clinical properties are also discussed. After each chapter is a list of the important references to the subject. Both a general index and an author's index are appended.

While the book is intended particularly for the student and laboratory worker by whom it should be heartily welcomed, it may well be utilized by the physician who desires to gain an understanding of these new therapeutic and diagnostic agents which seem destined to occupy a prominent place in the medicine of the future.

This book can be recommended as a useful, authoritative, and comprehensive survey.

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*The Chemistry of the Hormones. By Benjamin Harrow, Ph.D., Associate Professor of Chemistry, College of the City of New York, and Carl P. Sherwin, M.D. Dr. P. H. Cloth, pp. 227. The Williams and Wilkins Co., Baltimore, Md.

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EDITORIALS

Skilled Labor?

WITHOUT doubt the first ostrich to thrust his head in the sand in order to hide from his enemies must have been greatly surprised on withdrawing it, to find them all about him licking their chops in ghoulish anticipation.

The medical profession today finds itself in a somewhat similar predicament, for its head, so to speak, has long been buried in the beautiful tributes paid to it in the literature of the past, such as the familiar homage of Robert Louis Stevenson and the less familiar but impressive saying of the philosopher Descartes who wrote: "If ever the human race is raised to its highest practicable level, intellectually, morally and physically, the science of medicine will perform that service."

Having basked in the pleasant glow of such approbation for lo! these many years, it is somewhat disconcerting to the physician now to find himself

accused of entire responsibility for the fact that disease, its aftermath, and its management connote expense; and to observe, also, that the management of disease, heretofore thought properly to be his business, is now the target for all manner of "experts" to shoot at, let the arrows fall where they may even to the making of a Roman holiday, as it were.

As long as there are human beings there will be injury and disease to incapacitate them, and as long as this is so some arrangement must be made to take care of them. These facts are incontestable but, somewhat strangely, what these measures shall be, how they shall be inaugurated, and in what manner they shall be carried out, are matters which are passing, if they have not already done so, from the hands of the medical profession.

On second thought this is not so strange, after all, for the medical profession has had no small share in the development of this situation. The problem itself, which recently has come to be spoken of as "medical economics," is as old as the profession but any discussion of it in the past has been somewhat frowned upon as somewhat undignified and tainted by commercialism; and despite its importance, immediate and personal, many have become somewhat weary of the apparently endless and, it must be said, sometimes pointless discussions of it without which few county society meetings can nowadays be held.

No one denies that the subject is one well worthy of discussion and concerted action but that concerted action toward a clear-cut and definite end will be initiated by the medical profession itself the experience of the past leads one to doubt, first, because medical economics has never before been the subject of frank discussion by physicians, and second, because, very probably, few doctors realize that the profession itself is in no small measure responsible for the situation in general. Perhaps a still further reason may be in the fact that the medical profession has long been obsessed with its ideals whereas the solution, if there be one, of the present problem will depend, not upon ideals, but upon what are usually spoken of as business principles, and the doctor has long prided himself upon being no business man.

The doctor, with some justice, may resent the bland usurpation of the right to regulate his profession by the various philanthropic, economic, efficiency, and other experts who appear to have taken over the job but there is little reason, after all, for excessive surprise at their appearance. For medicine has been preparing the way for them for centuries and has been building within itself a Frankenstein, although until present times there has been reluctance to recognize this and a tendency to smother the occasional voice which now and then has been heard crying in the wilderness.

Few, indeed, of the thousands of young men graduated in medicine have ever heard a word indicating that, while medicine is a profession of vital importance and inherent dignity, it is also a business, the business of controlling disease and, incidentally, of making a living for those who practice it.

There is nothing impossible in the association of an ideal with a business just as there is no disgrace in professing openly that one is earnestly engaged

in making an honest living but this latter aspect of medicine has somehow been kept under cover as being in some fashion incompatible with professional ideals.

And so, although responsibility for the care of the sick and indigent is not an individual but a community responsibility, the community has managed to saddle the doctor with the heaviest share of the load, as shown by the growth and expansion of hospitals and their activities until they are now actively engaged in the competitive practice of medicine.

Only recently has the doctor begun to realize this and he has yet to fully appreciate in some measure that it has come to pass and been made possible solely because of his connivance and active assistance without which, indeed, it could not be possible.

Poverty and disease are inescapable ills and certainly the responsibility for their existence is not primarily the physician's. Nevertheless when the poor are sick the doctor has to answer for them as if he were responsible for them.

Exposure and lack of food leading to lessened resistance are admitted factors in the incidence of disease. Even so, no one expects the builder to supply houses, the grocer to furnish food, or the clothier to hand out overcoats.

When disease occurs the community through taxes and contributions has furnished hospitals to which the poor can go.

The doctor, too, has through taxes and contributions also contributed to their upkeep, but it has not always been clearly appreciated that without his additional time-consuming, laborious, and personal services hospitals would be just buildings of no use per se to the sick.

Oddly enough, the public in general, even the intelligent public, is largely ignorant of the fact that physicians are not remunerated for the time spent in hospital free wards and dispensaries. Nor do even the doctors appreciate that, according to the statistics of one large city, 81 per cent of doctors are on hospital staffs and 67 per cent devote an average of seven or eight hours per week to dispensary work entirely without remuneration. Moreover, at least 35 per cent of patients receiving free care are not really entitled to it.

Although the business of treating disease is the function of the doctor, as it is conducted in the hospital, he has little or no control over how it shall be done and little if any voice in its management. The lay Board of Managers decides upon the rate to be charged in "free wards," money allocated to the hospital entirely, and decides who may or may not be charged for the professional services rendered. The lay social service worker decides whether or not a dispensary patient shall receive treatment.

Although it is the inalienable right of every physician to decide who shall be treated free, this is decided for him by clerks, not always efficient, of the social service departments subservient to lay boards of trustees and the superintendent, seldom a physician.

Hospitals are largely lay managed and lay controlled and the doctor is fast becoming merely the skilled labor employed by them, with this differ-

ence: that unlike all other forms of skilled labor he works for nothing and, apparently, likes it.

So hospitals have grown to mammoth industries based upon the unrecompensed, unrecognized, and unappreciated work of the doctor who now finds them actively engaged in competition with him through the insurance schemes now advocated and partly in practice and the indiscriminate abuse of their free facilities.

It is small wonder that, in view of the fact that when the cost of medical care is analyzed the cost of *hospital care* bulks large indeed, that the doctor —a few of him, at least—is beginning to wonder if the worm might not venture a turn or two. At least, so they think in Australia where very recently a movement was inaugurated to secure concrete monetary recompense for the hospital work heretofore done for nothing by the doctor to his own gradually increasing, and now acute, disadvantage.

It would do hospitals and their managements no harm to consider just how they would carry on without the skilled assistance rendered freely by the doctor. The picture would be at least interesting.

—*R. A. K.*

The Bacteriologic Study of Food Handlers

ONE of the modern prophylactic provisions designed to lessen the incidence of diseases in the spread of which the carrier is regarded as of importance, is that which calls for the bacteriologic study of food handlers, a regulation which may be said, without injustice, to be more honored in the breach than in the observance.

Certain it is, however, that whenever an outbreak of enteric disease occurs there is a common tendency to demand a bacteriologic survey of all food handlers or persons engaged in other occupations which might render them a menace.

In view of the time, the labor, and the expense involved, it is well worth while to consider the results achieved, to balance these two factors, and to ascertain if the results warrant the effort expended.

Memory recalls an instance somewhat to the point which occurred during the World War.

In one of the infantry regiments of a certain division withdrawn from the line for rest, refitting, and replacement, there occurred a few cases diagnosed as typhoid fever and which, of course, were of interest. The history of the outbreak revealed that, despite the general orders to the contrary, this regiment on its way back from the front had neglected consistent chlorination of drinking water and had used the raw, untreated and commonly polluted waters of the French countryside. This information, coupled with the knowledge that antityphoid vaccination had not always been completed in the raw troops hastily sent overseas, seemed to furnish an explanation of the occurrence of a few cases of typhoid fever. But the powers that be

decreed that a search for carriers must be made and, accordingly, two officers, twelve enlisted men and a complete portable laboratory outfit traveled from the Central Medical Department Laboratories at Dijon to a small village "somewhere in France" and cultured an entire regiment at that time 1,800 strong.

The net result was the discovery of one carrier of *B. paratyphosus B.*, picked up while with the National Guard on the Mexican border.

The moral of this tale, if any, is that there may be more to the study and control of enteric outbreaks than isolated bacteriologic studies, to which fact attention has recently been called by Gilbert and Coleman[1] in discussing the examination of food handlers in state hospitals after outbreaks of typhoid fever had occurred in two.

In one hospital the examination of 116 specimens from 53 food handlers was without result. In the other, two carriers of *B. typhosus* and one of *B. paratyphosus B.* were found after the examination of 2,053 specimens from 813 food handlers.

The next result of the examination of 2,169 specimens, therefore, was the detection of three carriers one of whom, from a consideration of all the evidence, was probably not a carrier but recovering from an unrecognized infection.

As Gilbert and Coleman comment, assuming that, when large numbers are handled the average cost of an examination for *B. typhosus* is about $2.00, the total cost involved in the detection of one carrier under the circumstances above, was $1,500.

As they pertinently remark, since by the ordinary methods of examination of food handlers involving the study of one or two specimens every carrier will not be picked up, and since even when extensive studies are carried out only a relatively small percentage of positive findings will be encountered, a false sense of security results.

It should be emphasized, repeatedly, if necessary, that a study of environmental factors, careful history-taking and a thorough survey of all the factors accompanying and *preceding* the outbreak is an essential sine qua non in the study of enteric outbreaks. Not only is the information thus obtained of great value in itself, but, in addition to that fact it may be secured without extraordinary expenditure.

Moreover, it greatly enhances the value of the bacteriologic studies by enabling a selection of those to be examined, namely those who have had enteric disease, colitis, cholecystitis, etc., or whose immediate associates have had typhoid or paratyphoid infections.

Such a program, of course, is subject to modification as, for example, when cases of enteric disease occur along a milk route or among the patrons of a restaurant. In such a case, when the carrier is not found among those with a suggestive history, *where he should first be looked for*, then all the food handlers concerned should be examined.

When the outbreak under study suggests dysentery the problem is still further complicated by the fact that, as has recently become evident, organisms not included in the well-known dysentery group and heretofore regarded as nonpathogenic may be of etiologic importance.

Welch and Mickle,[2] for example, have recently reported the occurrence of dysentery-like diseases apparently caused by *Shigella alkalescens* and typhoid-like diseases accompanied by invasion of the blood stream by intestinal bacteria ordinarily without etiologic relationship to disease have been reported by others.

Bacteriologic surveys are too often ordered or deemed advisable without previous thorough studies of the outbreak which at times may definitely restrict the number of those to be examined while at the same time increasing the effectiveness of the laboratory study, and very often without a full realization of the expenditure of time, labor, and money involved.

West, Borman, and Mickle[3] in analyzing the examination of milk handlers over a period of seventy-seven months report that the total cost involved in the conduct of 91,257 examinations was $48,048; the average cost of detecting each of the 71 carriers found being $677.

While the per capita cost per consumer per annum was less than two cents in 1933, the cost per carrier varied from year to year, from a minimum of $387 to a maximum of $3,843.

It is profitable to consider, as suggested by Gilbert and Coleman, the more effective results to be expected if similar sums were expended in the pasteurization of products wherever possible, the improvement of sanitary facilities, the provision of training in personal hygiene, the removal from work and the treatment of food handlers who are obviously ill, the careful study of epidemiologic factors, and the examination of series of specimens from individuals *whose history or clinical manifestations warrant it.*

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3. West, D. E., Borman, E. K., and Mickle, F. L.: The Detection of Carriers Among Food Handlers in Connecticut, Am. J. Pub. Health **24**: 493, 1934.

—*R. A. K.*

CORRESPONDENCE

Dear Sir:

I have read the article, "The Bactericidal Power of Blood," by Dr. Reuben Ottenberg and find that his results are not only at variance with those observations published by me in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, October, 1933, but they do not agree with the authors cited in that paper. His conclusions appear, also, to be not in accord with that part of Cecil's technic which deals with the clotting of the blood in the isolation of streptococci from the blood stream.

The technic used by Dr. Ottenberg is open to criticism as mentioned by Topley and Wilson, "The Principles of Bacteriology and Immunity," Vol. I, page 170, and it may be that the results obtained were due to this technic.

Topley and Wilson (same page, etc.) state, "Other organisms, for instance most staphylococci and streptococci, are not killed by normal or by immune sera." Dr. Ottenberg's findings do not agree with this.

Recently, I found *Br. abortus* alive after three months in a defibrinated blood culture. At the time of obtaining this culture, the serum had an agglutination titer of $\frac{1}{2000}$. The culture had been kept at 35° C.

FREDERICK W. SHAW.

MEDICAL COLLEGE OF VIRGINIA,
RICHMOND, VA.,
MARCH 5, 1934.